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• ANNALES CRYPTOGAMICI et PHYTOPATHOLOGICI •
Volume VI

# PATHOGENIC
# FUNGI

# ANNALES CRYPTOGAMICI
# et PHYTOPATHOLOGICI

*(incorporating Annales Bryologici)*

*edited by*

FRANS VERDOORN, Ph.D.

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> *Wij en konnen den Heer en maker van het geheel Al niet meer verheerlijken, als dat wij in alle zaken, hoe klein die ook in onse bloote oogen mogen zijn, als ze maar leven en wasdom hebben ontfangen, zijn al wijsheit en volmaaktheit, met de uiterste verwondering sien uit steken.*
>
> *Antoni van Leeuwenhoek*

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*1947*

*WALTHAM, MASS., U.S.A.*

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# BIOLOGY of PATHOGENIC FUNGI

*edited by*

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# *FOREWORD*

*The discovery of a species of* Botrytis *in muscardine, of* Achorion *in favus and of* Monilia *in thrush were probably the first demonstrations that micro-organisms caused disease. In the century or more since these observations our knowledge of pathogenic mycology has been extended but interest in this subject has been overshadowed by that in bacteriology and virology, and progress in these apparently more difficult fields has been far greater.*

*Our understanding of fungus infections has been and still is hampered by many deficiencies. We lack exact criteria and methods of identification. We need improved methods of isolation and propagation. We are ignorant of the pathogenic potentialities of many fungi found in lesions, e.g. the* Hyphomycetes *and species of* Monilia *isolated from pulmonary disease. We have little knowledge of the sources of sporadic mycoses. We know something about poisoning by* Amanita *but our information as to allergic diseases caused by non-parasitic fungi is fragmentary. For most of the severe mycoses we have no effective therapy. Immunization and chemotherapy which control so many bacterial infections have made little headway against the fungi.*

*Progress in medical mycology has been due chiefly to observations of clinicians more interested in host reactions than in the parasites causing them. The studies which have been made on the fungi have been almost entirely descriptive and largely morphological. These phases are important. Nothing is more fundamental to an understanding of mycotic diseases than accurate identification of the causative agents. Identification of the* Fungi imperfecti *presents real difficulties demanding meticulous objective study and probably attack by new methods. These difficulties have, however, led many writers to neglect observation and indulge in verbose controversies as to nomenclature which have led not to clearness but only to added confusion. What fruitful observations have been made are obscured by this sterile overgrowth of arguments about terminology. At present the literature of pathogenic mycology is a taxonomic morass quite forbidding to the serious student of infectious disease.*

*The absurd commotion over terminology has obscured the fact that significant information as to the biology of some pathogenic fungi is already available. In the present volume Dr.* NICKERSON *has collected summaries by pioneers in different phases of the subject. It records what has been accomplished and indicates more emphatically how much needs to be done. One may see in the bibliographies that these studies have been made by botanists, chemists, and physicians, with their reports so scattered in various journals that they may well have escaped the attention of many to whom they would be helpful. One sees also that practically all were published in the past fifteen years and the majority quite recently, which is a hopeful indication of increasing activity in the field.*

*This is the sort of information we need but we need more of it. The solution of our practical problems can hardly be found without more basic knowledge. Problems of the war brought innumerable reminders of the fact that basic research is essential to practical scientific advance. The outbreak of scrub typhus found entomologists who knew the carrier ticks and were ready with information essential to control of the disease. The appearance of schistosomiasis found experts on snails similarly at hand.*

*The discovery of penicillin brought a sudden need for mass production of this fungus metabolite. The need could hardly have been met had there not been available the studies of* THOM *on classification of the* Penicillia, *the formulae of* RAULIN and of CZAPEK, *and many more recent studies of fungus nutrition, and at the Northern Regional Research Laboratories* COGHILL, MOYER *and a group of associates with a mine of information on the metabolism of fungi. Already on file in the Peoria collection was a strain of* Penicillium *which proved capable of producing workable amounts of penicillin in submerged culture, a property lacking in* FLEMING'S *strain. Even more essential were the principles and techniques developed by organic chemists which made possible purification and concentration of the product. The rapid supply of fantastic amounts of potent penicillin seemed miraculous but the miracle was made possible by long trains of basic research, much of which seemed to have no practical application through the years in which they were accomplished.*

*All of which gives hope that research in the biology of the fungi will lead to solution of the many practical problems in medical mycology. It would be futile to attempt to predict what studies will solve which problems, for the essence of fundamental research is that it seeks truth and not its applications. However there are already indications of what may happen. The specific deficiency in thiamin which* ROBBINS *found in* T. discoides *may prove reliable in its identification. Knowledge of this had already proved useful in the isolation of this species in the hands of* GEORG. LEWIS *and* HOPPER *have shown that glucose is essential to the production of certain characteristic pigments and* BENHAM *and* HAZEN *have found that special nutrients induce the formation of spore forms by which species are defined. Studies along these lines promise to solve many problems in identification.* NICKERSON *has already made therapeutic application of results of his studies on respiration of the fungi. It is certainly to be expected that information as to the chemical constituents and metabolites of these fungi will clarify problems in pathogenicity and immunity.*

*The volume in hand will interest all those concerned with problems in medical mycology and will furnish investigators a useful summary of what has been accomplished in the fields summarized. The editor and contributors are to be congratulated on completion of a helpful and difficult task.*

J. G. HOPKINS, M.D.

*New York City,*
*Spring, 1947*

# *PREFACE*

*Our aim in this volume has been to discuss some aspects of the biology, physiology, and biochemistry of the fungi pathogenic for man. The volume is not meant to be a clinical manual nor has an attempt been made to consider all the fungi of medical importance. It has been our hope that by bringing information from several fields to bear on the pathogenic fungi a volume useful to students of this difficult group would result.*

*Where possible, a comparative presentation has been attempted for the pathogenic fungi and other microorganisms. Aside from the theoretical interest in such comparisons, it may be possible in some instances to transfer to investigations on the fungi causing disease in man information, or procedures that have yielded valuable information, from allied fields of endeavor.*

*The recent world conflict served to focus attention on inadequacies in our knowledge of the mode of infection, methods of transmission, means of prevention, and specific treatment of fungus diseases, particularly diseases of the skin. Several chapters in this volume point out* lacunae *in our knowledge on these topics. The increase in success of control of diseases caused by bacteria following the accumulation of information under the broad heading "biology of bacteria" permits one to hope that similar success may attend the extension of comparable (but not necessarily identical) investigational approaches to the fungi. From the relatively small volume of pertinent research that has appeared on the pathogenic fungi, we have indications of some promise.*

*The editor wishes to thank the contributors for their cooperation in this work—for their advice and encouragement as well as for their contributions. It is a pleasure to acknowledge the invaluable assistance of the general editor of this series, Dr.* FRANS VERDOORN, *and to thank for their advice and help Drs.* JOHN E. ANDERSON, LAURENCE IRVING, J. R. SCHOLTZ, J. G. HOPKINS, K. V. THIMANN, WILLIAM H. WESTON, *and* JOHN G. DOWNING. *Vignettes at the ends of chapters were kindly done by* JOHN B. CHADWICK; *the assistance of* H. K. NICKERSON *with the mss. and figures for four chapters is deeply appreciated. To the publishers of several journals who granted permission to use copyrighted material our thanks are expressed; acknowledgment is made individually in each instance.*

THE EDITOR

*Norton, Mass.*
*June, 1947*

> "TOUT CE QUI EST GÉNÉRALEMENT COMMUN AUX VÉGÉTAUX ET AUX ANIMAUX, COMME TOUTES LES FACULTÉS QUI SONT PROPRE À CHACUN DE CES ÊTRES SANS EXCEPTION, DOIT CONSTITUER L'UNIQUE ET VASTE OBJET D'UNE SCIENCE PARTICULIÈRE QUE N'EST PAS ENCORE FONDÉE, QUI N'A PAS MÊME DE NOM ET À LAQUELLE JE DONNERAI LE NOM DE *Biologie.*" (Chevalier DE LAMARCK, *Philosophie Zoologique,* 1809).

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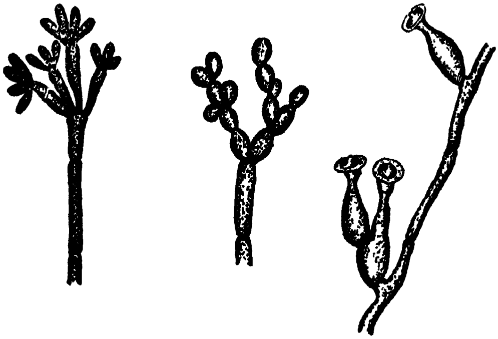

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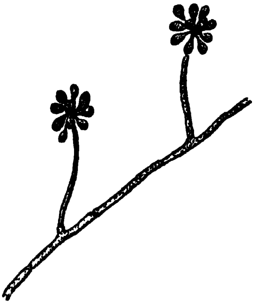

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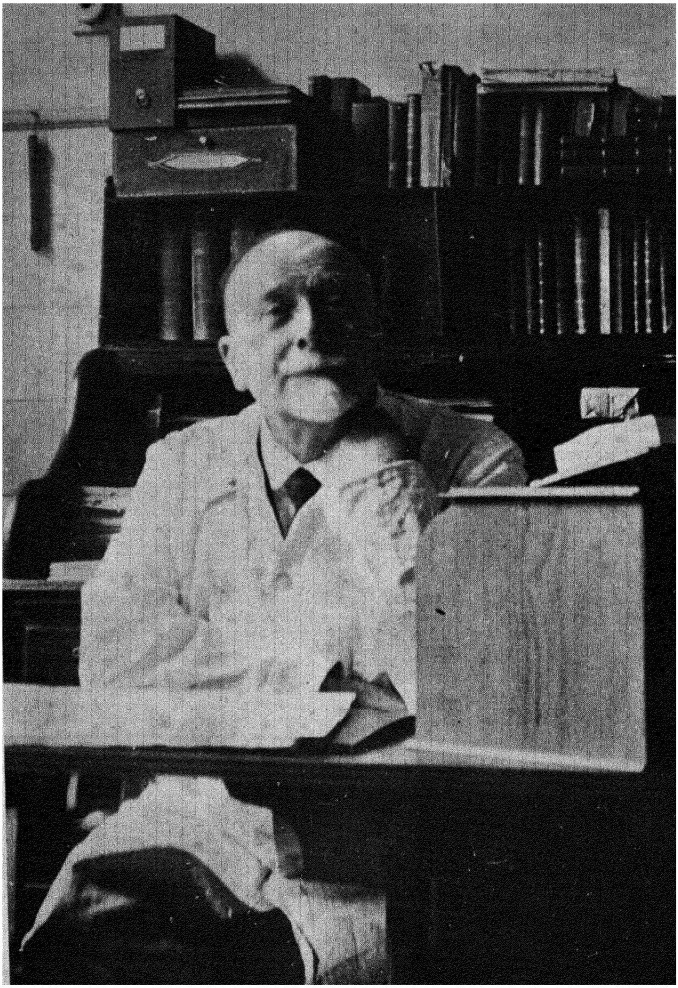

RAYMOND JACQUES SABOURAUD

(1864-1938)

Pioneer mycopathologist,
at his desk in the Saint-Louis Hospital, Paris,
in his 70th year. (*Photo by Dr. N. F. Conant*).

## Chapter 1

# INTRODUCTION

*by*

WALTER J. NICKERSON

Encompassed in a study of the biology of an organism may be information from any category that can contribute to a fuller understanding of the life of the organism. The group of fungi to which these pages are devoted possess in common the ability to parasitize man, causing clinically recognizable diseases. It is within our province, therefore, to consider these fungi as entities and in their relations to the host.

The localization of dermatophyte invasions to the superficial layers of the skin has been noted by many authors as a subject meriting extensive investigation. It would be very interesting to know something of the mechanisms involved in limiting growth of these organisms to the hair, nails, and epidermis. From *in vitro* studies on the nutrition of dermatophytes it is clear that these tissues of epidermal origin make no unique contribution in supporting fungus growth. Strains of *Trichophyton mentagrophytes* make abundant growth in a medium containing a single amino acid and inorganic salts—such apparently meager demands might be supplied by any tissue in the body. Viewed in another light one might consider that all tissues of the body actively resist infection by dermatophytes; all successfully so, except those epidermal structures mentioned, which are only partially successful. Experiments of BROCQ-ROUSSEU *et al.* (1926) and SULZBERGER (1929) on the injection of spore masses of species of *Trichophyton* and *Microsporum* into guinea pigs revealed no attack of internal organs. Injections by varied and multiple paths with simultaneous scarification (but not inoculation) of epidermis on the backs of guinea pigs were followed in two weeks by mycotic lesions of the scarified areas on the majority of the animals. Similar findings were reported by FRIED and SEGAL (1929) who showed it was possible to recover fungus spores from the blood within three days after inoculation; they supported the view that damage to dermal capillaries in the scarification process apparently enabled spores to reach the skin. In contrast to the resistance of most tissues to attack by dermatophytes there is no question about the susceptibility of epidermal structures to such infection. Though prone to such infections, it is clear that the dead epidermal structures are not without influence on fungal growth and may be considered to exert effects ranging from a restraint of growth to modifying influences on morphogenetic development of the fungus in the event of infection. The important demonstration by DAVIDSON and GREGORY

(1933, 1934) on the course of development of fungi contained in infected skin scales and hairs following removal of these structures to moist chambers leads one to suspect the existence of an *in vivo* influence on morphogenesis of the fungi. It is well known that the structures exhibited by fungi in skin scales and hairs are limited to mycelial strands and arthrospores[1]. Yet, when these infected hairs and scales were removed from the body, there developed within a very short time from fungal hyphae the elaborate spore forms and other morphological structures that had long been known to accompany growth of these organisms on artificial media. The nature of the suspected *in vivo* influence on morphogenesis has not been established; it would appear not to reside in the skin scales or hair and might be of a chemical nature supplied to the scales and hair by diffusion from adjacent, resistant tissues. While not proved, it seems unlikely that holding of the excised scales and hairs at high humidities could alone be responsible for the elaboration of the *"in vitro"* spore forms since some areas of the body which can be infected by dermatophytes are frequently held at near saturation for long periods without there developing the described fungal structures; such could be said for crural and intertrigonal infections, particularly in tropical climates.

That there is an actual restraint by the skin to growth of dermatophytes is clear. In any survey of large numbers of people in a similar population (such as at an army post) it is revealed that a considerable percentage is free from any evidence of mycotic infection, yet all may have been equally exposed to infection. Actually, in view of the luxuriant growth of dermatophytes that excised skin and hair will support at appropriate temperatures and humidities, it is a fair question to inquire how dead epidermal structures remain uninfected at all. The *p*H of the skin seems of minor importance with respect to dermatophyte infections, since these organisms grow readily from *p*H 3 to 10.5. There is some evidence, however, that resistance of the skin to fungus infection can be increased or decreased by alteration in the concentrations of chemical components through nutrition. In some experiments by BENHAM (1935) it was found that rats on vitamin deficient diets were more susceptible to infection by strains of *Torulopsis neoformans* and recovered less quickly than those on complete diets. Further, it is well known that less well nourished elements in a population show a higher incidence of several types of skin infections than do the better nourished elements (*see* chapter by MARTIN). That this could be no more than in part a result of the concomitant hygienic level is seen from accounts of native populations existing at different nutritional but comparable hygienic levels (*see* VAN ·VEEN, 1942). Most striking testimony on the importance of the nutritional level is that of Dr. M. A. DONK on the incidence of mycotic skin infections among war prisoners on Java. Shortly after internment, following the sharp break from a high nutritional level to one of bare subsistence, the incidence of infections was high among the prisoners. As time went on and the weight of all decreased continuously, infections cleared and the incidence became quite low, reaching a vanishing point after three years internment, by which time most of the prisoners had lost up to half of their original weight. On liberation and the return of sufficient food, fungus infections flared dramatically among nearly all who had been confined, to disappear slowly as an individual gained weight over a period of time. The survival of these people for three years of internment on a near-starvation diet holds many points that deserve the closest attention from all interested in nutrition.

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[1] **Typical fuseaux of *Microsporum lanosum* were recently found occurring in abundance on hairs of the scalp of a 16-months-old girl by Drs. B. APPEL and H. B. ANSELL. This unusual case is being studied further.**

It is known from the work of EGGLETON (1939) that loss of thiamin from foods (as in the polishing of rice) is frequently accompanied by a loss of other constituents, commonly metals. He found further that paralleling a deficiency of thiamin in cases of beriberi is a marked diminution of the concentration of zinc in human tissues and that, in general, the distribution of zinc in foods paralleled the distribution of thiamin. The procedures (synthesis by microorganisms) employed by the prisoners (*see* BAARS and BOGTSTRA, 1946) to add to the vitamin content of their polished rice ration would not replace minerals, though sedimented boiled river water was used to moisten the rice prior to inoculating it with the complex-cultures. It might well be, though it has not yet been demonstrated by analysis, that minerals (in particular zinc, cobalt, and manganese) might have been among the major deficiencies with respect to the balance of this low level diet.

It is a fact that the parts of the body affected in dermatophytosis are the regions of the highest zinc concentration in the body. EGGLETON has shown that normal, healthy human hair and nails average about 200 p.p.m. zinc, while epidermis averages about 100 p.p.m. and whole skin only 25 p.p.m. These values can be reduced by ½ in serious thiamin deficiencies. Zinc chloride in concentrations of only 1/100 molar (650 p.p.m.) have been found to inhibit the respiration of the dermatophytes almost 100% while 1/1000 molar (65 p.p.m.) caused inhibitions of 30% (NICKERSON and CHADWICK, 1946). The latter concentration is lower than the normal zinc content of hair or epidermis and suggests very strongly that the zinc content of these dead structures of epidermal origin may have a rôle in the defense against fungus invasion. Experimental evidence on this point must be obtained before further discussion is warranted.

The inability of the dermatophytes to infect tissues other than the skin seems to have been satisfactorily demonstrated as mentioned previously. The underlying basis for this inability has not been explained. From work on bacteria it is known that ability to invade the dermis is correlated closely with the elaboration by the bacteria of enzymes of the hyaluronidase type, often referred to as spreading-factors (*see* reviews by Duran-Reynals, 1942, and by Mann and Mann, 1944). It would be of some interest to learn of the abilities of the dermatophytes with respect to the elaboration of enzymes of this type.

In connection with the findings of Brocq-Rousseu and others mentioned previously on the production in scarified epidermal areas of mycotic lesions following intravenous injection of spore masses, the recent work of Black (1946*a, b*) on a similar situation with the wound-tumor disease of plants seems worthy of note. This virus disease (causal agent, *Aureogenus magnivena*) is remarkable in that many tumors develop at the loci of wounds distant from the point of inoculation. A tumor apparently does not develop at a site unless the site has been wounded (the phloem is implicated as the tissue which must be damaged). The virus appears to exhibit considerable tissue specialization in its effects. Perhaps the resemblances between this virus disease of plants and the experimentally induced mycotic infection of animals are completely superficial but the explanations offered to account for the infection of only damaged epidermis in the experimental mycoses are merely suggestive and attention to developments in this phytopathological problem might be profitable. It is known for example that one and the same fungus can cause disease in plants and in animals (*cf.* Benham and Kesten, 1932, on the transmission of Sporotrichosis).

Considerable attention has been given in the pages to follow to many quite recent investigations on the nutrition, metabolism, and chemical constituents of pathogenic fungi. In many instances confirmation of these reports has not yet appeared, but it has been attempted to present the information in a framework that may suggest investigations on fungi and infections incompletely known. One point emerging from this synthesis of information is the negative correlation between the nutritional requirements of a fungus and its pathogenicity. For the organisms for which data are available we might list, in approximate order of increasing pathogenicity, a few and compare their nutritional requirements:

| ORGANISM | REQUIREMENTS |
|---|---|
| *Pityrosporum ovale* | oleic acid, thiamin, amino acid nitrogen |
| *Trichophyton mentagrophytes* | amino acid as nitrogen source (some isolates require thiamin) |
| *Candida albicans* | biotin; can use ammonium nitrogen |
| *Coccidioides immitis* and *Blastomyces dermatitidis*[1] | no growth factors required; can use inorganic nitrogen |

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[1] Levine and Ordal (1946) have indicated that *B. dermatitidis* may be grown in a medium containing only minerals, glucose, and inorganic nitrogen. References and further discussion for the other organisms listed will be found in Chapter 9.

It will be noticed that nutritional requirements diminish with increasing pathogenicity. This situation is in marked contrast to that holding with pathogenic bacteria. KNIGHT (1938; *see* also DEN DOOREN DE JONG, 1942) distinguished five evolutionary stages among bacteria: *1*) autotrophic forms; *2*) heterotrophic forms utilizing inorganic nitrogen; *3*) bacteria requiring amino acid nitrogen; *4*) those with growth-factor requirements in addition to requirements for organic nitrogen; *5*) bacteria developing only in living organisms. No truly pathogenic bacteria are known for groups 1 and 2, and most of the pathogenic forms belong to group 4. Synthetic ability decreases, of course, from group 1 to group 5. Within a genus which contains both pathogenic and saprophytic species, the pathogenic forms invariably have more exacting nutritional requirements. With the development of these requirements there has evolved concomitantly the ability on the part of the bacterium to overcome in one way or another natural barriers to infection possessed by organisms. The development of infectious ability possesses survival value (in the evolutionary sense) for the exacting bacterium. With fungi there are no examples of autotrophic forms, so group 1 can be dropped from consideration, and no fungi causing disease in animals are known as obligate parasites, though there are whole groups of fungi causing disease in plants that would come under group 5 (and KNIGHT's scheme of pathogenicity vs. nutrition undoubtedly holds for the phytopathogenic fungi). Under the above grouping, *Coccidioides immitis* would be in group 2, most strains of dermatophytes in group 3, and *Pityrosporum ovale* in group 4.

The dermatophytes and *P. ovale* may be considered the most parasitic of the pathogenic fungi and, anomalously, among the least pathogenic. HENRICI (1940) has discussed this topic in detail; the dermatophytes have only occasionally been isolated as saprophytes from nature and *P. ovale* seems only to have been isolated from humans, where its pathogenicity is most questionable. On the other hand, *Coccidioides* may possibly occur in nature as a saprophyte (EMMONS, 1942). Too little is known about the pathogenic fungi to pursue these comparisons and speculations very far at the moment. It is clear that differences in the mechanism of pathogenicity exist between bacteria (endo- and exo-toxin formation here important) and the fungi. With bacteria there is considerable correlation between elaboration of toxins and complexity of nutrition while with fungi the few reports of the production of powerful toxins have been confined mainly to saprophytic species (*see* HENRICI, 1930).

The recent work of LANGERON (1945) "Précis de Mycologie" became available after this volume was in proof. It contains a brilliant chapter (78 pp.) with the provocative title, "A quoi se ramène la mycologie médicale?" that relates the fungi of medical importance to the overall view of mycology in its broadest aspects.

The problems enveloping the proper nomenclature and taxonomic position of the fungi to which clinical significance has been attached have called forth a voluminous literature (*see* review by EMMONS, 1940). Some consideration will be given to these problems in the chapters to follow, for a sound taxonomy is, practically, an essential basis for any comparative endeavor on a group of organisms. In general, the nomenclature will follow that employed by CONANT *et al.,* 1945). The attempt has been made to adhere to the International Rules of Botanical Nomenclature and to be guided by the results of international agreements where such have been reached. In cases of names regarded as synonyms, the name employed in the work cited will generally be given in parentheses. No new names or combinations are proposed, and support is given to a conservative treatment with respect to the dermatophytes, *Candida,* and other groups.

*Literature Cited:* —

BAARS, J. K. and J. F. BOGTSTRA. Natuurwetens. T. voor Nederl. Indië *102:* 135, 1946.
BENHAM, R. W. J. Infect. Dis. *57:* 255, 1935.
BENHAM, R. W. and B. KESTEN. J. Infect. Dis. *50:* 437, 1932.
BLACK, L. M. Nature *158:* 56, 1946*a*.
BLACK, L. M. Growth, Supplement, 1946*b*.
BROCQ-ROUSSEU, D., A. URBAIN and J. BAROTTE. C. R. Soc. Biol. Paris *95:* 966, 1926.
CONANT, N. F., D. S. MARTIN, D. T. SMITH, R. D. BAKER and J. L. CALLAWAY. Manual of Clinical Mycology. Saunders, Philadelphia, 1945.
DAVIDSON, A. M. and P. H. GREGORY. Nature *131:* 836, 1933.
DAVIDSON, A. M. and P. H. GREGORY. Can. J. Res. *10:* 373, 1934.
DONK, M. A. Personal communication, 1946.
DEN DOOREN DE JONG, L. E. Nederl. T. voor Geneeskunde *86:* 734, 1942.
DURAN-REYNALS, F. Bact. Rev. *6:* 187, 1942.
EGGLETON, W. G. E. Biochem. J. *33:* 403, 1939.
EMMONS, C. W. Botan. Rev. *6:* 474, 1940.
EMMONS, C. W. Pub. Health. Repts. *57:* 109, 1942.
FRIED, S. M. and M. B. SEGAL. Arch. Dermatol. and Syphilol. Chicago *19:* 98, 1929.
HENRICI, A. T. Molds, Yeasts, and Actinomycetes. Wiley & Sons, New York, 1930.
HENRICI, A. T. J. Bact. *39:* 113, 1940.
KNIGHT, B. C. J. G. Bacterial Nutrition. Med. Res. Council Special Rept. Series, No. 210, London, 1938.
LANGERON, M. Précis de Mycologie. Masson et Cie, Paris, 1945.
LEVINE, S. and Z. J. ORDAL. J. Bact. *52:* 687, 1946.
MANN, T. and C. LUTWAK-MANN. Ann. Rev. Biochem. *13:* 25, 1944.
NICKERSON, W. J. and J. B. CHADWICK. Arch. Biochem. *10:* 81, 1946.
SULZBERGER, M. B. Arch. Derm. Syph. Wien *157:* 345, 1929.
VAN VEEN, A. G. Ann. Rev. Biochem. *11:* 391, 1942.

## *Chapter 2*

# ON THE BIOLOGY OF THE PATHOGENIC TORULOPSIDOIDEAE

*by*

J. LODDER and A. DE MINJER

**Introduction:** — In discussing the biology of the pathogenic *Torulopsidoideae* it is desirable in the first place to give a definition of this subfamily and in the second place to decide which of the members of the group are to be considered as pathogens.

The subfamily *Torulopsidoideae*[1] belongs to the family *Torulopsidaceae*. This family comprises the greater part of the asporogenous yeasts. Those asporogenous yeasts which are characterized by the formation of a red pigment of carotenoid nature are brought together into another family, the *Rhodotorulaceae*. Two subfamilies belong to the *Torulopsidaceae*, the *Torulopsidoideae*, in which are collected the species which do not form a pseudomycelium, and the *Mycotoruloideae*, containing the species which are characterized by the formation of a pseudomycelium. The following genera belong to the *Torulopsidoideae: Torulopsis, Pityrosporum, Mycoderma, Kloeckera, Trigonopsis*, and *Schizoblastosporion.*

It is not easy to decide which of these *Torulopsidoideae* are to be considered as pathogens. It is well known that many times on isolating yeasts from human or animal tissue or from feces, one gets the impression of dealing with a casual contamination. For instance the yeast can even live and multiply in the body without being responsible for a manifest disease process. On the other hand it is known that species which generally are recognized to be pathogens may be isolated from sources such as fruit juices where they have been growing saprophytically. According to HENRICI (1940) all fungi which cause deep-seated mycoses normally live as saprophytes. Among these deep-seated mycoses he reckoned also the "blastomycosis" which is caused by *Torulopsis neoformans.* That such dangerous organisms are so seldom isolated as free living from nature results from the fact that they are only rarely searched for, and that the isolation may be rather difficult.

From the above it is clear, on the one side, that isolation of a yeast as free living from nature does not mean that it cannot be a pathogen; on the other side, that by no means all yeasts isolated from human or animal sources have to be considered as pathogens.

To be perfectly sure about the presence of any pathogenicity in a yeast species, animal tests are indispensable. But only a few species have been examined in this way. With most species, however, such an investigation seems quite superfluous as there is not the slightest indication of any pathogenicity.

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[1] Throughout this paper the taxonomy as outlined in the monograph of LODDER (1934) will be adhered to.

We shall in what follows consider only those *Torulopsidoideae* to be pathogens for which the pathogenicity has been experimentally proved.

**Pathogenicity of the Torulopsidoideae:** — Several scientists have studied the pathogenicity of yeast species by means of animal tests. We mention NEUMAYER (1891), RAUM (1891), RABINOWITSCH (1896), SKCHIWAN (1899), FOULERTON (1900), GREENBAUM and KLAUDER (1922), TANNER and DACK (1924), WHITE (1927), WHITE and SWARTZ (1928), FINNERUD (1929), SKOLNIK (1930), BECKER and RITCHIE (1930), and BENHAM (1935). It was, however, not possible to establish the identity of most of the yeasts studied by these authors because of the rather incomplete descriptions given in most cases. Several of the strains studied seem to belong to the sporogenous yeasts or to the *Mycotoruloideae.* Unquestionably there were also species of *Torulopsidoideae* among them.

With the exception of *Torulopsis neoformans* (usually designated by one of the numerous synonyms for this yeast) pathogenicity has not been proved for any of these species. This need not surprise us if we consider the various representatives of the *Torulopsidoideae* more systematically in connection with their pathogenicity. For we can at once eliminate from this discussion a large number of these organisms; there is nothing to suggest any pathogenic action of the species belonging to the genera *Mycoderma, Kloeckera, Trigonopsis* and *Schizoblastosporion.*

The species of the genus *Pityrosporum* are generally associated with a skin disease "pityriasis capitis." BENEDEK (1930), however, denied the pathogenic character of these yeasts. The skin diseases may only create favorable conditions for the development of these organisms. Moreover, this genus has been dealt with elsewhere in this book. We therefore will omit it from further discussion.

Neither are most of the species of *Torulopsis* known to be pathogenic. There is only one species for which pathogenicity is certain, *viz., Torulopsis neoformans,* which causes a chronic infectious disease, mainly of the central nervous system, known as "torulosis" or "Torula infection" or "blastomycosis of the central nervous system."

There are a few *Torulopsis*-species, it is true, which were isolated from human or animal sources and which have been suspected of being pathogens, as for instance, *Torulopsis rotundata.* This organism was isolated from a case of pulmonary tuberculosis (REDAELLI 1925), however its pathogenicity has never been submitted to an experimental test.

In this connection we should also mention *Torulopsis glabrata.*

This organism is repeatedly and exclusively isolated from human sources (ANDERSON 1917, BENHAM 1935, LODDER and DE VRIES 1937, BLACK and FISHER 1937). Several investigations have shown, however, that this organism is probably not a pathogen.

BENHAM studied this species together with some other *Torulopsis* species. In extensive animal tests she found the pathogenicity of all these yeasts — with the exception of *Torulopsis neoformans* — to be most doubtful.

LODDER and DE VRIES studied five strains of *Torulopsis glabrata.* All strains were of human origin. Five rats were injected intracardially with suspensions of each strain. Five of the 25 injected rats died after about three months. Three of these had been injected with a strain isolated from the sputum of a girl 25 years of age who remained for several weeks in a feverish condition in a hospital. From an X-ray examination the case had been diagnosed as pulmonary tuberculosis. The fourth dead rat had been injected with a strain which had been isolated from an ulcer on the vulva of a girl 17 years old suffering from ulceric stomatitis. The strain which had been injected into the fifth dead rat was isolated from normal human feces. The yeast could be cultivated from the lungs of the dead rats but, of course, this is no proof for the pathogenicity of this yeast. After an intercardial injection yeast cells can easily enter into the lungs. It is no wonder that under especially favorable conditions they can grow there without causing pathological changes. It is noteworthy that it was the "sputum" strain which showed the best development. Possibly this strain was, because of its origin, already more or less adapted to the conditions existing in the lungs.

BLACK and FISHER (1937) isolated *Torulopsis glabrata* from the pharynx and from the sputum in a case of bronchopneumonia in a child. They injected the yeast intraperitoneally into rats. One rat was killed after five days. The omentum was contracted and contained a few hard nodules. The yeast was cultivated from all the organs. A second rat was killed after ten days and showed the same pathological changes. This time *Torulopsis glabrata* was found in the spleen, the omentum, the kidney, the adrenals and the fluid of the pleural cavity. With a culture obtained from these animals another rat was inoculated, which was killed after three weeks. The omentum again was contracted into a hard mass. In this animal the yeast was found only in material from the omentum, the kidney and the peritoneal fluid.

According to these observations *Torulopsis glabrata* may possess some pathogenicity. However, it should be taken into account that the time of observation in the animals was rather short and a definite decrease of the virulence was undeniable.

DE MINJER again examined *Torulopsis glabrata* together with a number of other species belonging to the genus *Torulopsis, viz., T.*

*flavescens, T. Laurentii, T. liquefaciens, T. luteola, T. Molischiana,* two strains of *T. albida* and an unidentified *Torulopsis*-strain. One strain of *T. albida* was of human origin (sputum); all the other strains had been isolated as sapropytes from nature. These yeast strains were tested in connection with a study of *Torulopsis neoformans,* with which species they are more or less closely related in morphological respects.

De Minjer first injected a number of mice intracranially. More than a month later the animals were killed. They showed no lesions and neither in the brain nor in the other organs could yeast cells be observed. Since this path of inoculation gave no results, de Minjer injected a suspension of each strain into a number of mice and rats both subcutaneously and intraperitoneally. But no pathological changes could be observed with these animals; yeast cells were never found in any of their organs. It seems therefore to be certain that these strains were not pathogens.

In striking contrast with all these uncertain or negative results are the numerous successful infections with *Torulopsis neoformans.* The greater part of these experiments have been done with strains isolated from "torulosis" patients. Usually the investigators aimed at a demonstration of a causal relation between the yeast and the observed lesions of the tissue. As a rule these strains had been isolated shortly before they were tested. Benham, however, studied strains of human origin, which had been cultivated for many years on different artificial media. The virulence of these strains, which were injected peritoneally into young rats, was at first slight but after some passages in animals it was greatly increased.

Strains isolated from the skin or from the intestines of normal individuals showed no virulence whatever. Fisher and Arnold (1936) could confirm these observations.

De Minjer studied a strain which had been isolated from a patient with "torulosis". This strain had been injected into mice and guinea pigs a short time after it had been isolated from the patient. After that the strain had been cultivated on artificial media for more than five years. De Minjer injected suspensions of this strain intracranially into mice and guinea pigs. He used two different cultures; a freshly transferred culture and a culture which had been transferred five years before and had dried up very much.

Both cultures caused changes in the animals which were of the same kind as those obtained after injection of the strain a short time after its isolation from the patient. The virulence of the strain had not decreased. With other strains of this yeast de Minjer obtained the same results, though sometimes the changes developed much more rapidly and extensive metastases in other organs were observed. Production of extensive metastases was not the case with the first strain mentioned. Consequently the virulence was different for the various strains.

The investigations discussed here have all been performed with strains of *Torulopsis neoformans* of human origin. It also seems important to consider the results obtained with strains isolated as free living from nature. Only two cases of such isolations are known. SANFELICE (1894, '95, '96 '97, '98, 1903) cultivated a strain from fruit juice and KLEIN (1901) from milk. SANFELICE injected pure cultures into the abdomen of guinea pigs; the animals died after a short time and the injected yeast could be recovered; apparently it had multiplied rapidly within the animal.

This survey makes it clear that there is only one yeast species in the *Torulopsidoideae, viz., Torulopsis neoformans,* which has unquestionably pathogenic properties. From this it follows that a discussion on the biology of the pathogenic species of the *Torulopsidoideae* can be restricted to a discussion of the biology of *Torulopsis neoformans.*

**Historical Survey of Torulopsis neoformans:** — *Torulopsis neoformans* has often been dealt with in the medical mycological literature. It would take us too far afield, however, to give a complete historical survey of *Torulopsis neoformans*. We will confine ourselves to the most important facts.

The organism was described for the first time in 1894 in Germany by BUSSE (1894, 1895). He discovered it in a subperiostal lesion of the tibia of a woman. He succeeded in isolating the organism and in cultivating it in different media. He also transferred parts of the diseased tissue into animals. The organism seemed to multiply and new lesions appeared. BUSSE knew the organism to be a yeast but he did not name it. Later he referred to it as a *Saccharomyces* species. It was only in 1910 that VUILLEMIN named it *Saccharomyces hominis.*

The second description also dates back to 1894 and comes from Italy. SANFELICE isolated the organism from fermenting fruit juices. This, and the isolation from milk by KLEIN are, as far as is known to us, the only cases in which this organism has been isolated free in nature and not from human or animal sources. SANFELICE named this organism *Saccharomyces neoformans.* He gave it this name because he considered it as a possible cause of tumors. It was his opinion that the organism was closely related to the one described by BUSSE.

The organism was described by STODDARD and CUTLER (1916) who had isolated it from the brain and the spinal cord of man. They named it *Torula histolytica,* because their observations seemed to indicate that the yeast cells dissolved tissues. Since then the organism has been observed about 70 times almost everywhere in the world and has been described under very different names.

At first it was not known that the organisms described under so many different names all belonged to one species. LODDER, however, after a comparative study of the many *Torulopsis* strains present in the collection of the yeast division of the "Centraalbureau voor Schimmelcultures" at Delft, noticed in 1934 that 26 strains labelled with different names (among which were five different specific names) all belonged to one and the same species.

SANFELICE was the first to give this organism a specific name, REDAELLI (1931) the first to classify this organism in the genus *Torulopsis;* consequently the organism must be designated as *Torulopsis neoformans* (SANFELICE) REDAELLI.

BENHAM (1935) studied 22 strains of this organism which she received partly as named strains, partly as unidentified strains. Among the identified strains different species were represented, but all were proved to belong to the same species. BENHAM, however, named this organism *Cryptococcus*[2] *hominis.*

Until 1936 no spore-formation had ever been reported for this organism, but in this year TODD and HERRMANN (1936) described the formation of an ascus in which one spore developed. They observed this spore-formation with different strains. Their description is as follows: In old cultures two types of cells were observed. One type was fairly thick-walled, the other thinner-walled. Sometimes both types were seen with tubes of varying lengths. On two occasions a kind of conjugation was observed between the two types of cells. Both cells had short tubes by which they became united. The contents of the thinner-walled cell passed through the short connecting tube into the thicker-walled cell. In numerous pairs of cells observed the thinner-walled cells were noted in various stages of collapse. The thicker-walled cell became composed of a central oval or spherical area surrounded by one or two outer layers. The first of these layers was in contact with the inner mass except for a small area near one pole. This opening gradually widened, leading to a complete separation between outer layers (ascus wall) and inner mass (spore). In the inner mass (spore) several spherical bodies appeared, which took fat stains. The inner mass (spore) could bud, even while the husks were still attached. In consequence of these observations the authors classified this yeast into the sporogenous genus *Debaryomyces* and named it *Debaryomyces hominis.*

REDAELLI, CIFERRI, and GIORDANO (1937) confirmed these observations. They retained this organism in the genus *Debaryomyces,* however, because of priority — under the name of *Debaryomyces neoformans.*

We shall refrain here from further discussion of these findings, and refer to the matter later in this article.

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[2] At that time the generic name *Cryptococcus* had been often accepted in the medical world to indicate asporogenous yeasts. We are, however, of the opinion that it should be avoided in yeast taxonomy as it is both a "nomen dubium" and a "nomen confusum" (LODDER, 1937).

The above gives only a short survey of the history of *Torulopsis neoformans*. As has been said before, much literature has appeared about this organism. An impression of the enormous number of synonyms under which this yeast has been described can be obtained from a publication by GIORDANO (1938) who gives a list with no less than 68 synonyms!

**Symptomatology and Pathology of Torulosis:** — *Torulopsis neoformans* was first found as the cause of disease in 1894 by BUSSE in a tumorlike lesion of the tibia. The typical symptoms of torulosis were first described by STODDARD and CUTLER in 1916.

The pathological changes in torulosis are found mostly in the meninges and in the cerebrum. Besides an affection of the central nervous system there are sometimes lesions containing yeast cells in many other organs. These lesions, however, cause only few symptoms and in such cases the clinical picture is also predominantly characterized by the lesions of the central nervous system.

The nature of the clinical symptoms of torulosis is dependent on these lesions. General symptoms indicating an infection, *e.g.*, fever, leucocytosis, and increased sedimentation rate of erythrocytes, are not or only slightly evident.

The most important clinical symptoms are due to an increased intracranial pressure. Besides, there are generally meningeal symptoms (stiffness of the neck, sign of Kernig), while lesions in the brain tissue may cause neurological symptoms.

The increased intracranial pressure causes a violent headache, vomiting, and diplopia, owing to paresis of the nervus abducens. The headache generally dominates the clinical picture. Sometimes for a long period it is the only symptom patients show. For instance the patient of RAPPAPORT and KAPLAN (1926) has long been known as "the man with the headache." The pain is always violent and at times so vehement that the patient screams out loud.

The disease can be diagnosed by microscopic investigation of the cerebrospinal fluid, in which the typical yeast cells are always present in large number. The size and shape of the yeast cells in these circumstances are rather different from those of cells grown in culture media. The yeast cells cannot easily be stained but the capsule can be observed very well in India ink preparations and can be stained with thionin.

The pathological changes are characterized by the presence of large numbers of yeast cells. Sometimes, especially at the base, the brain is covered with a gelatinous mass, consisting exclusively of yeast cells. The peculiar gelatinous quality is due to the capsules of the yeast cells.

In cases of long duration the meninges show a chronic inflammation with epitheloid cells, lymphocytes, leucocytes, and giant cells. In this granulation tissue there are numerous intra- and extracellular yeast cells. In acute cases tissues do not show any reaction.

The yeast penetrates the brain via the perivascular spaces. The spaces are widened by the growing yeast. Finally the perivascular membrane ruptures, after which there is a rapid distribution into the brain substance. This is disintegrated by the growing yeast cells and cavities filled with yeast cells are formed. In the case described by LONGMIRE and CAMPELL GOODWIN (1939) these cavities were so big and numerous that they at first thought they had to do with foamy brains.

Sometimes the brain tissue surrounding the foci shows a slight reaction of the glia cells (FREEMAN 1931); mostly however there is no reaction whatever. In some cases the yeast cells are distributed by the blood and cause metastases in other organs. In the brain, too, new lesions may arise in this way. In cases where lesions in the central nervous system are absent, torulosis is especially observed in the lungs and in the skin. Here, too, the clinical and anatomical symptoms are completely dominated by the large numbers of yeast cells.

The prognosis of torulosis is absolutely fatal. All cases known up to now have ended in death. An effective therapy is not known. As symptomatic treatment frequent lumbar punctures are indicated, which have a favorable influence on the violent headache. Antibodies in the blood serum and in the spinal fluid are found in only a few patients with torulosis, so that there is not much prospect for a serum treatment. Nevertheless, a more detailed serologic investigation in torulosis would be desirable.

**Epidemiology and Pathogenesis of Torulosis:** — The way in which infection with *Torulopsis neoformans* takes place is still a great problem. The disease occurs only sporadically. In animals, too, some sporadic cases have been observed. Though most cases have been described in America, cases of torulosis have been met in all parts of the world. Thus the geographical distribution of the disease throws no light upon the etiology.

Torulosis might originate because a wide spread organism, under special circumstances, may be the cause of disease. With most torulosis patients, however, we find no circumstances which might have favored the infection, while on the other side *Torulopsis neoformans* has hardly ever been found apart from pathological processes. Besides we have seen earlier that the virulence of the yeast can be preserved under very adverse circumstances. So the organism can cause infection as soon as it comes into contact with man or animal. However, there still remains the question: where in all the separate cases has the yeast come from? Further investigation will be necessary to make this clear. It would therefore be of special importance to confirm the few older observations regarding the occurrence of *Torulopsis neoformans* as occurring free in nature.

Nor has the question been solved as to how the yeast enters the body and reaches the central nervous system. It is generally assumed infection takes place through the nasopharynx or the lungs, though this has never been proved.

If infection has taken place, *Torulopsis neoformans* causes peculiar lesions in the tissues, as a result of the large numbers of yeast cells produced. In the cerebrospinal fluid there is a very luxurious growth of the yeast. Hence the marked increase of the intracranial pressure. This is very evident in small laboratory animals, *e.g.* mice, where the whole cranium may expand considerably.

In the organs, the growing yeast form large cavities by compressing the tissues. Dissolution of tissue, as originally reported by STODDARD and CUTLER, never takes place (DE MINJER). As a rule the tissues do not show a marked inflammation. Only in cases of long duration is a granulation tissue formed, identical to that induced by foreign bodies. In our opinion the lesions in torulosis arise exclusively mechanically. The clinical symptoms also point to this, as they are principally caused by the considerably increased intracranial pressure, while toxic symptoms are lacking.

Causing disease in such a way is exceptional with pathogenic microrganisms. Only in anthrax do some of the symptoms also develop mechanically. The large numbers of bacilli occurring in the blood in anthrax block the capillaries.

A second point of similarity is that both *Torulopsis neoformans* and *Bacillus anthracis* possess a capsule. Now the chemical investigation of the capsule of the anthrax bacillus has given a remarkable result, for it consists partly of a polypeptide compound which is wholly built up by a so-called unnatural[3] amino acid, *viz.*, d-(—) glutamic acid (BRUCKNER and IVÁNOVICS 1937). For such compounds the higher organism does not have any proteolytic enzymes, so that they cannot be broken down by the host. The capsule of the anthrax bacillus therefore makes the bacterial body unassailable by the host, and rapid and luxurious growth can take place uninterruptedly.[4]

With so much similarity between *Torulopsis neoformans* and *Bacillus anthracis* the question arises: does the capsule of these yeast cells also contain specific chemical compounds?

In this respect it seems of much importance that ASCHNER,

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[3] With a few exceptions there occur in nature exclusively amino acids with the same stereo configuration (*l*-series). In so-called "unnatural" amino acids there is a reverse configuration (d-configuration). The prefix d-(—) used here has been derived from the new nomenclature; in their original publications BRUCKNER and IVÁNOVICS made use of the old designations.

[4] On this thought is based the theory of KÖGL (1939) about the growth of tumor cells, the proteins of which should also contain d-amino acids.

MAGER, and LEIBOWITZ (1945) found that the capsule of *Torulopsis neoformans* is, at least under special conditions, characterized by the fact that it is stained blue by the addition of iodine. These investigators report that at least two different polysaccharides may be present. It seems not unlikely that in these capsules, too, specific compounds are formed which afford protection to the yeast cells growing in the tissue. This would then explain the almost unlimited growth of the yeast. Apparently the tissues, and especially those of the central nervous system, form an excellent culture medium. So perhaps chemical investigations may explain why *Torulopsis neoformans* is able to cause disease and why the greater part of the yeasts have no pathogenic properties.

The reaction of the tissues occurring some time after infection may be identical with the inflammation by foreign bodies; in tuberculosis an important part of the histological lesions is also attributed to foreign body action.

**Morphological and Physiological Characteristics of Torulopsis neoformans:** — We shall give here a survey of the morphological and physiological characteristics of *Torulopsis neoformans.* The methods used here to determine these characteristics are the same as have been applied by LODDER in her study of the asporogenous yeasts.

*Morphological characteristics.* — In young cultures cells are round to slightly oval. There are many budding cells. The average cells are 4-6$\mu$ in size. The cells are surrounded by a capsule, which is poorly developed in young cultures. In old cultures, however, the capsule is much more marked. Often the cells are filled with refractile bodies, which stain with Sudan III. The size of the cells in the tissues may differ rather markedly from that in culture media. A normal formation of ascospores, as generally occurs in sporogenous yeasts, has not been observed by us. We could confirm only in part the observations of TODD and HERRMANN. We have never seen conjugation of cells.

The description of the life-cycle of this organism as given by TODD and HERRMANN is rather different from what one usually observes in sporogenous yeasts. In the first place a collapse of a part of the zygote is rather unusual, and the ascus belongs to a very unusual type. Generally when spore formation is completed the ascus is a mere sack containing only one or more spores lying loose in the ascus and are not grown together with the wall of the ascus. TODD and HERRMANN, however, observed in *Torulopsis neoformans* a spore which sheds its container. This, of course, is quite a different thing.

Although the observations of TODD and HERRMANN have been confirmed by REDAELLI, CIFERRI and GIORDANO, by GIORDANO (1938) and by HENRICI (1941) we still feel hesitant in accepting these phenomena as criteria for spore formation. We do hope that a closer study may throw more light upon these questions. But even should it appear that we really have to do with ascospore formation (TODD and HERRMANN mentioned, too, the desirability of an investigation of nuclear phenomena), this would be an ascospore formation of a very special type, differing from that of the other sporogenous yeasts.

*Cultural characteristics.* — In liquid media such as malt extract or peptone broth with dextrose, there is a strong bottom growth. A mucoid ring, and in old cultures, a thin mucoid pellicle is occasionally formed.

On solid media such as malt agar streak cultures, the growth is creamy to yellow-brown, smooth, shiny and nearly always mucoid. HENRICI (1941) reports the occurrence of a light tan color with a rosy cast on 5 per cent dextrose, 1 per cent Bacto-peptone agar; the color appears in cultures within a few days.

*Physiological characteristics.* — There is not much known about the nature of the capsule. As far as is known to us, only ASCHNER, MAGER and LEIBOWITZ (1945), have made a study of the capsule, establishing the presence of at least two different polysaccharides. Under special culture conditions, an acid *p*H of the medium being especially important, amylose has been indicated as one of these polysaccharides on grounds of its reaction with iodine, and through comparison with the spectrophotometric data for the complex of iodine with pure potato amylose. The organism does not ferment any sugar. The following sugars are assimilated: glucose, fructose, mannose, galactose, saccharose, maltose, lactose. The following nitrogenous compounds are assimilated: ammonium sulphate, asparagine, urea, and peptone; potassium nitrate is not assimilated. Rather good growth is obtained in a synthetic medium with ethyl alcohol as the sole source of carbon.

**Taxonomy of Torulopsis neoformans:** — Following our survey of the literature which has appeared on this organism, and summary of its characteristics, a final word may be said regarding the systematic place of the organism.

If it should appear that this yeast really is a sporogenous yeast, as TODD and HERRMANN suggest, we still cannot accept its classification into the genus *Debaryomyces*. As we have already stressed there exists a real difference between the type of spore formation described by these authors and the method known for the true ascosporogenous yeasts. In the presumed case it would, therefore, be desirable to create a special genus for this organism.

But so long as the life-cycle as described by these authors is not more satisfactorily proved, we must consider the organism to be an asporogenous yeast. It then belongs to the family of *Torulopsidaceae*. Since the formation of a pseudomycelium never has been observed, it must be placed in the subfamily *Torulopsidoideae*.

On liquid media a pellicle is not produced promptly, and when it has developed it is not a dry one. Therefore the organism does not belong to the genus *Mycoderma.* Most of the cells are round or slightly oval. Consequently the organism belongs to the genus *Torulopsis.* As *"neoformans"* was the first specific name given to it, it has to be designated as *Torulopsis neoformans* (SANFELICE) REDAELLI.

**Summary:** — From a discussion of the investigations of several authors on the pathogenicity of the yeasts belonging to the subfamily *Torulopsidoideae,* it is clear that only one species has unquestionably pathogenic properties, *viz., Torulopsis neoformans.* A historical survey of this yeast has been given.

The disease caused by this yeast is a chronic infectious disease, mainly of the central nervous system, known as "torulosis" or "Torula infection" or "blastomycosis of the central nervous system." Yeast cells develop mainly in the cerebrospinal fluid and in the brain where they cause a violent pressure. As a consequence of this, a bad headache is the most important clinical symptom. The disease has proved to be fatal in all cases.

The question of how the yeast enters the body and reaches the central nervous system has not been solved.

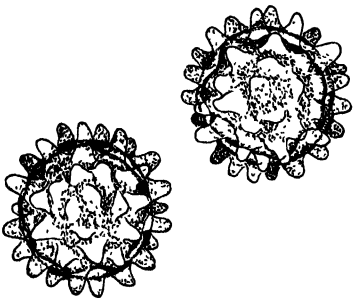
It seems not unlikely that in the capsule which surrounds the yeast cell specific compounds are formed which make the yeast cell unassailable by the host.

A description of the morphological and physiological characteristics of *Torulopsis neoformans* has been given. The interpretation of the observations on this yeast by TODD and HERRMANN as ascospore formation has been criticized. The systematic place of *Torulopsis neoformans* has been discussed.

*Literature Cited:* —

ANDERSON, H. W. J. Infect. Dis. *21:* 341, 1917.
ASCHNER, M., J. MAGER, and J. LEIBOWITZ. Nature *156:* 295, 1945.
BECKER, S. W. and E. B. RITCHIE. Arch. Dermatol. Syphilol. *22:* 790, 1930.
BENEDEK, T. Zentrlblt. f. Bakt. I, *116:* 317, 1930.
BENHAM, R. W. J. Infect. Dis. *57:* 255, 1935.
BLACK, R. A. and C. W. FISHER. Am. J. Dis. Child. *54:* 81, 1937.
BRUCKNER, V. and G. IVÁNOVICS. Zeit. f. physiol. Chem. *247:* 281, 1937.
BRUCKNER, V. and G. IVÁNOVICS. Naturwiss. *25:* 250, 1937.
BRUCKNER, V. and G. IVÁNOVICS. Zeit. Immunitätsforsch. *90:* 304; *91:* 175, 1937.
BUSSE, O. Zentrlblt. f. Bakt. *16:* 175, 1894.
BUSSE, O. Arch. Path. Anat. *140:* 23, 1895.
FINNERUD, C. W. Arch. Dermatol. Syphilol. *20:* 454, 1929.
FISHER, C. V. and L. ARNOLD. Univ. Illinois Bull. *33,* no. 51, Ill. Med. Dent. Monogr. *1,* no. 3, 1936.
FOULERTON, A. G. R. J. Path. Bact. *6:* 37, 1900.
FREEMAN, W. J. Psych. Neurol. *43:* 236, 1931.
GIORDANO, A. Mycopathologia *1:* 274, 1938.
GREENBAUM, S. S. and J. V. KLAUDER. Arch. Dermatol. Syphilol. *5:* 332, 1922.

HENRICI, A. T. J. Bact. *39:* 113, 1940.
HENRICI, A. T. Bact Revs. *5:* 97, 1941.
KLEIN, E. J. Hyg., *1:* 78, 1901.
KÖGL, F. and H. ERXLEBEN. Zur Atiologie der malignen Tumoren. Amsterdam, 1939.
LODDER, J. Verhand. Kon. Akad. Wet. (Tw. Sectie), *32:* 1, 1934.
LODDER, J. Mycopathologia *1:* 62, 1937.
LODDER, J. and N. F. DE VRIES. Mycopathologia *1:* 98, 1937.
LONGMIRE, W. P. and T. CAMPELL GOODWIN. Bull. Johns Hopkins Hosp. *64:* 22, 1939.
DE MINJER, A. Onderzoekingen over Torulosis en over de pathogeniteit van gist. Diss. Utrecht, 1941.
NEUMAYER, J. Arch. f. Hyg. *12:* 1, 1891.
RABINOWITSCH, L. Zeit. f. Hyg. *21:* 11, 1896.
RAPPAPORT, B. Z. and B. KAPLAN. Arch. Path. *1:* 720, 1926.
RAUM, J. Zeit. f. Hyg. *10:* 1, 1891.
REDAELLI, P. I miceti come associazione microbica nella tubercolosi pulmonare cavitaria. Pavia, 1925.
REDAELLI, P. Riv. d. Biologia *13:* 3, 1931.
REDAELLI, P., R. CIFERRI and A. GIORDANO. Boll. Sez. Ital. Soc. Intern. Microbiol. I-II, 1, 1937.
SANFELICE, F. Ann. Ist. Igiene R. Univ. Roma *4:* 1894.
SANFELICE, F. Ann. Ist. Igiene R. Univ. Roma *5:* 1895.
SANFELICE, F. Zentrlbt. f. Bakt. I, *17:* 113, 625; *18:* 521, 1895.
SANFELICE, F. Zeit. f. Hyg. *21:* 32, 394, 1896.
SANFELICE, F. Zeit. f. Hyg. *22:* 171, 1897.
SANFELICE, F. Zeit. f. Hyg. *29:* 463, 1898.
SANFELICE, F. Zeit. f. Hyg. *44:* 364, 1903.
SKCHIWAN, Ann. Inst. Pasteur, *13:* 770, 1899.
SKOLNIK, E. A. Arch. Dermatol. Syphilol. *22:* 642, 1930.
STODDARD, J. L. and E. C. CUTLER. Monog. Rockefeller Inst. Med. Res. *6:* 1, 1916.
TANNER, F. W. and G. M. DACK. Zentrlbt. f. Bakt. I. *91:* 282, 1924.
TODD, R. L. and W. W. HERRMANN. J. Bact. *32:* 89, 1936.
VUILLEMIN, P. Rev. Gén. d. Sci. *12:* 732, 1901.
WHITE, C. New York State Med, *27:* 1116, 1927.
WHITE, C. and J. H. SWARTZ. Arch. Dermatol. Syphilol. *18:* 692, 1928.



### *Chapter 3*

# CHROMOBLASTOMYCOSIS AND ITS ETIOLOGIC FUNGI

*by*

ARTURO L. CARRIÓN and MARGARITA SILVA

## CLINICAL ASPECTS

**Definition:**— Chromoblastomycosis is a chronic, infectious, apparently non-contagious skin disease confined most frequently to one of the lower extremities and characterized clinically by the formation of nodular, verrucous, or cauliflower-like lesions. The infection may be caused by several species of dematiaceous fungi.

**Synonymy:**— The term chromoblastomycosis, first used by TERRA *et al.* in 1922, is not only a long word, but a misnomer falsely ascribing the disease to a *Blastomyces* and conveying the erroneous impression of an extraordinary color element in the clinical picture. Notwithstanding this, we believe that, for the present, this name should be preserved. It has been extensively used in the literature and it expresses the frequent resemblance of the disease to true blastomycosis. Futhermore, the prefix "chromo," though misleading in regard to the clinical picture, is descriptive of the etiologic fungus.

The following synonyms have been used or suggested for this disease: *"blastomycose negra"* (PEDROSO, 1911, cited by FONSECA and LEÃO, 1930); *"figueira"* (RUDOLPH, 1914); *"dermatite verrucosa por Phialophora verrucosa"* (PEDROSO and GOMES, 1920); *"formigueiro"* (GOMES, 1921); chromomycosis (MOORE and ALMEIDA, 1935); *"dermatite verrucosa cromomicósica"* (REDAELLI, 1935); *"dermatitis verrucosa blastomicótica"* (BOGGINO, 1937); Pedroso's disease, Fonseca's disease or Gomes' disease (WEIDMAN and ROSENTHAL, 1941); Pedroso and Carrión's disease (BARROS-BARRETO, 1943). Some of the synonyms just mentioned are almost as long or longer than chromoblastomycosis and are still misnomers; others might be criticized for bearing personal names. If the term chromoblastomycosis were to be changed at all, it should be done at an International Congress where the subject could be thoroughly discussed and settled by general agreement.

**History:**— It is generally accepted that chromoblastomycosis was discovered in Brazil. In 1911, PEDROSO, of São Paulo, noted the presence of large, dark-brown to yellowish, spherical bodies in a biopsy from a patient with nodular and ulcerated skin lesions of the foot and leg (PEDROSO and GOMES, 1920). He suspected a mycotic infection and was able to isolate from the lesions a dark colored fungus. The disease became known as *"blastomycose negra"* (black blastomycosis) in PEDROSO's laboratory (PEDROSO, quoted by FONSECA and LEÃO, 1930), but the study of the presumptive causative agent was postponed and the discovery was not reported until 1920 (PEDROSO and GOMES).

In 1914, RUDOLPH published his observations on a skin disease popularly known as *"figueira"* in Minas Geraes and Goyaz. The excellent clinical description and the mycologic findings given by RUDOLPH in his report clearly indicate that he was dealing with the same disease observed by PEDROSO in São Paulo three years before.

In 1915, MEDLAR and LANE, in the United States, published two elaborate communications on "A Cutaneous Disease Caused by a New Fungus . . ." in a patient from Boston. The infection in this patient had lasted for only a year and the clinical picture was not particularly specific; but the pathologist discovered a blastomycetic type of lesion with numerous, spherical, pigmented fungus cells, and the cultures revealed the presence of an organism producing a dark mycelium. The fungus was carefully studied and was named *Phialophora verrucosa.* The term *Phialophora,* representing a new genus (THAXTER, cited by MEDLAR, 1915) means cup-bearer and is descriptive of the conidiophores produced by the fungus in culture, while *verrucosa* indicates the type of lesion induced by the parasite on the human skin.

From the descriptions of MEDLAR and LANE, it was apparent that the infection in the Boston patient was identical in nature with that previously observed in Brazilian patients by PEDROSO and by RUDOLPH. This fact was recognized by PEDROSO and GOMES in 1920 when they published a report on four cases of the disease in Brazil, including the original case found by PEDROSO in 1911. In their report, PEDROSO and GOMES referred to the malady as a form of *"dermatite verrucosa."* It was not until two years later that the term chromoblastomycosis was first used to designate the infection (TERRA *et al.,* 1922).

In 1922, BRUMPT established that the organism responsible for PEDROSO's original case was not *Phialophora verrucosa* but a new species which he named *Hormodendrum Pedrosoi: Hormodendrum,* because some of the conidia observed in culture were produced in branching chains; *Pedrosoi,* to honor the investigator who discovered the disease. In the following year FONSECA and LEÃO conducted careful morphologic observations on eight fungus isolates from Brazilian cases and arrived at the conclusion, like BRUMPT, that the Brazilian organism was not a *Phialophora;* but they classed it in the genus *Acrotheca* because they found that the outstanding feature of the species was not the *Hormodendrum* sporulation already observed by BRUMPT, but the production of terminal spore clusters of characteristic *Acrotheca* type. Consequently, they renamed the fungus *Acrotheca Pedrosoi* (FONSECA and LEÃO, 1923). In 1936, NEGRONI, who made a mycologic study of another case of chromoblastomycosis occurring in Argentina, confirmed the presence of both the *Hormodendrum* and

*Acrotheca* types of sporulation in his patient's isolate. Considering that neither of the two genera above mentioned could legitimately admit a fungus showing the sporulating habits of the other, NEGRONI created the genus *Fonsecaea* to include organisms possessing the double method of sporulation characteristic of *Pedrosoi* and the name of the species was changed, accordingly, to *Fonsecaea Pedrosoi* (BRUMPT, 1922) NEGRONI, 1936, *comb. nov.* Finally, in September 1935 (CARRIÓN and EMMONS), and later in 1936 (EMMONS and CARRIÓN, p. 703), it was demonstrated that *Fonsecaea Pedrosoi* possesses, in addition, a semiendogenous type of sporulation by which conidia are produced in phialides. This observation, not only added a new and fundamental character to the species, but established its relationship with a third generic group, namely *Phialophora* MEDLAR, 1915.

In June, 1935, and in the following year, one of us (CARRIÓN, 1936), described in a Puerto Rican case, a third etiologic agent for chromoblastomycosis. The Puerto Rican fungus is generally a *Fonsecaea* with its characteristic triple method of sporulation, but it possesses sufficiently distinct qualities to warrant its registration as a new species which bears the name *Fonsecaea compactum* (CARRIÓN, 1935) CARRIÓN, 1940, *comb. nov.*

It is convenient to state at this moment that, after the publications of MEDLAR and LANE in North America and of PEDROSO and GOMES in Brazil, interest in chromoblastomycosis became increasingly progressive; year after year new cases of the disease were discovered, not only in Brazil and the United States, but in many other regions throughout the world; and by the end of 1945, at least 151 legitimate cases had been recognized. With the publication of these cases many supposedly new etiologic species were described and a long and confusing list of specific names was added to the mycology of chromoblastomycosis. Careful comparative studies (CARRIÓN, 1940 and 1942) have demonstrated that the large majority of these names are synonymous with *Fonsecaea Pedrosoi;* but there is no doubt that some of the newly described species, which have not been generally recognized as yet, are worthy of consideration and will be included in our discussion on "Mycologic Aspects" (*q. v.*).

**Incidence and Geographic Distribution:**— During the thirty-five years that have elapsed since the discovery of the first Brazilian case, the existence of chromoblastomycosis has been recognized in other parts of South America, in North and Central America, the West Indies, Europe, Africa, the East Indies, Japan, and Australia, leaving Continental Asia as the only part of the world as yet without reported cases. A careful review of the literature for this period has revealed a large number of infections classed as chromoblastomycosis, but the information given for some of these infections is not considered sufficient to establish their authenticity beyond doubt. It is only in 151 of the reported cases that the diagnosis appears to have been made on a sound basis and this number may be accepted as an approximate index of the recognized authentic cases of the disease during the last 35 years. The geographic distribution of this group of cases is as follows: Cuba, 43; Brazil, 41, South Africa, 12; United States, 9; Venezuela, 9; Puerto Rico,[1] 7; Russia, 5; Costa Rica, 4; Dutch East Indies, 4; Japan, 3; Algiers, 2; Argentina, 2; Australia, 2; and Rhodesia, Dominican Republic, Guatemala, Canal Zone, Mexico, Canada, Paraguay, and Uruguay, 1 case each. By plotting these cases on the map, it will be found that 119, or 79 per cent, occurred in tropical or subtropical regions, and only 32, or 21 per cent, in the temperate zones. According to these observations, chromoblastomycosis might be considered as a cosmopolitan disease, but its distribution would be predominantly tropical. This was to be expected from the fact that we are dealing with a fungus infection and it is well known that fungi generally thrive better in warm, moist climates.

**Symptomatology:**— The disease usually affects one of the lower extremities. It begins as a small papule or warty growth which may develop anywhere on the extremity, but is located, as a rule, on some part of the foot, whence the infection spreads upward through the gradual development of satellite lesions. The course of the pathologic process is extremely slow and the clinical history often reveals that the infection had existed for ten or more years at the time of examination.

In a typical, well advanced case of chromoblastomycosis the foot and leg are generally swollen and somewhat elephantiasic in appearance (Fig. 1). The lesions occur in great numbers, especially toward the distal portion of the extremity, and they are conspicuously varied in morphology. For the purpose of description it is convenient to class them into five different types, namely, the nodular, the tumorous, the verrucous, the plaque, and the cicatricial types.

The nodular type includes the youngest and smallest elements in the clinical picture and consists of moderately elevated, fairly soft, dull-pink to violaceous growths, the surface of which may be smooth, verrucous or scaly.

Through further development many nodules are gradually transformed into lesions of the second, or tumorous type. This type is represented by much larger and prominent, distinctly papillomatous, sometimes lobulated, tumor-like masses, partly or wholly covered with dirty gray epidermal débris, crusts, and horny particles. On the foot and lower leg, where the pathologic process tends to be most exuberant, the tumor masses often reach enormous dimensions, taking on the characteristic appearance of cauliflowers.

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[1] We have unquestionable evidence of the existence of eight additional authentic cases in Puerto Rico. These cases are not included in the above statistics because they have not been officially published yet.

In the third, or verrucous type of lesion, hyperkeratosis is the outstanding feature; the efflorescences are warty in appearance and may resemble *verruca vulgaris.* Growths of the verrucous type are frequently encountered along the borders of the foot.

The plaque type is the least common of the lesions of chromoblastomycosis. It consists of fairly flat, slightly elevated, variously sized and shaped areas of infiltration. They are reddish to violaceous in color, superficially scaly, and some of them show exaggeration of the lines of cleavage. The development of small, papillomatous vegetations, or larger nodules, within a plaque is sometimes observed and may lead to great variations in the morphology. When present in the clinical picture, the plaques are generally found on the higher portions of the extremity and never on the lower leg or foot.

Finally, the cicatricial type of lesion is represented by growths that enlarge by peripheral extension while healing takes place at the center with the production of sclerotic or atrophic scarring. Cicatricial lesions may cover more or less extensive areas and are usually annular, arciform or serpiginous in contour.

The lesions of chromoblastomycosis develop slowly but progressively and, in the course of time, a few or many of them may coalesce to form extensive and often bizarre aggregates. The infected tissues are easily traumatized and bleed readily. When pressed with the fingers many of the lesions discharge a whitish, caseous material and sometimes fluid pus at one or more points. Secondary bacterial infections and ulceration frequently complicate the clinical picture and are mostly responsible for the foul smelling character of the eruption, a feature that is usually perceptible at a distance from the patient. Subjectively, pruritus may be an important symptom and some patients complain of pain. In advanced cases there is partial or total incapacity for work.

The deeper tissues are not usually involved. The lymphatic glands draining the diseased focus may participate in the process, but this is not the rule. However, adenitis due to bacterial complications is not infrequent. Metastases through the blood stream appear to be extremely rare, but there is no question that they can be produced (CARRIÓN and KOPPISCH, 1933). Finally, no systemic symptoms have yet been recorded from the infection.

It should be emphasized that the dermatologic picture just given is a general representation of the symptomatology in well developed, typical cases of chromoblastomycosis of the lower extremities. Although location in one of the lower extremities is the rule, the disease may affect the skin in almost any other part of the body, either as aberrant lesions produced during the course of a limb infection, or as the original site of attack. Involvement of the upper extremities ranks second in order of frequency, the initial lesion being usually located somewhere on the hand or wrist, although it may appear in any other segment of the limb. Infection of the face has been recorded in three instances, while the neck and trunk have been involved only once each and in different cases.

In chromoblastomycosis, as in any other chronic, infectious dermatosis, the clinical type of the infection in individual patients may vary in accordance with the duration of illness, the degree of virulence of the infecting parasite, the nature of the host reaction, and the location of the pathologic process. The possible correlation of clinical types with one or another of the specific parasites producing the disease has been suggested by various workers, but our present knowledge is too limited to warrant final conclusions on this phase of the subject.

**Histopathology:**— Chromoblastomycosis falls in the group of infectious granulomas. The pathologic process affects both the cutis and the epidermis, and it develops toward the surface with little or no tendency to involve the deeper structures. Infection is apparently extended to adjacent regions by autoinoculation through the epidermis, or by way of the superficial lymphatics (CARRIÓN and KOPPISCH, 1933; MERIIN, 1938; EDSON DE ALMEIDA, 1942; BRICEÑO-IRAGORRI, 1939, cited by AZULAY, 1944). Metastases through the blood stream are very rare but may be produced (CARRIÓN and KOPPISCH, 1933).

When the lesions are examined microscopically, the epidermis is generally thickened and often folded to fit the underlying papillomatous elevations (*see* Fig. 2). The thickening is due largely to hyperplasia of the *stratum Malpighii* which not only is broader than normal but shows irregular growths penetrating more or less deeply into the cutis. These growths may be sufficiently pronounced to resemble an epithelioma of the prickle cell type. The *stratum corneum* shows marked hyperkeratosis and is often distorted. Polymorphonuclear leucocytes are sometimes noted infiltrating the epidermal layers and, not infrequently, they form miliary, abscess-like accumulations.

In the cutis the pathologic reaction is essentially granulomatous with a varied cellular infiltrate consisting of lymphocytes, plasma cells, large mononuclear leucocytes, polymorphonuclear leucocytes, eosinophiles, Russell's fuchsin bodies, epithelioid cells, and occasional giant cells of both the Langhans' and foreign body types. The infiltrate may be focal or diffuse. In many places it is distinctly tuberculoid (Fig. 3). Miliary abscesses are frequently observed, but widespread necrosis, softening and free suppuration are not so common. The lesions of chromoblastomycosis show a constant tendency toward fibrosis as a natural defense mechanism to wall off the infective process. This tendency becomes especially noticeable in the older foci of infection where the major part of the lesions often consists of fibrous tissue.

The most significant character in the pathologic picture is undoubtedly the presence of the infecting fungus (Fig. 3). This may be seen as rounded, occasionally crescent-shaped, often septate bodies measuring about ten microns in diameter, with fairly thick and dark cell walls and a coarsely granular protoplasm possessing a pigment which has been variously described as ochre, olivaceous, yellowish-green and dark chestnut. The parasitic cells may occur singly or in groups. In the cutis they are variously located within giant cells, free in the tissues and, not infrequently, in the center of microabscesses; but they may be found also within epithelial pearls and microabscesses in the epidermal layers. Evidence of germination is often noted in the *stratum corneum.*

**Etiologic Factors:**— Chromoblastomycosis occurs most frequently during the period of active adult life. Among 109 authentic cases of the disease in which the age was recorded, there were 2 patients who contracted the infection during the first decade of life; 8 were infected during the second decade; 23 during the third; 29 during the fourth; 25 during the fifth; 13 in the sixth; 6 in the seventh, and 3 in the eighth. According to these records it is evident that the highest incidence of the disease lies between the ages of 20 and 50 years (ca. 71 per cent of the cases). It may be added that the extreme ages at which infection took place were 3 (TSCHERNJAWSKI, 1929) and 76 (TAKAHASHI, 1937, p. 53) years respectively.

Chromoblastomycosis is decidedly more common among males. A review of 138 authentic cases in which the sex was registered, showed that 132 were males, a proportion of 96 per cent.

There seems to be no race immunity. However, in a collection of 124 clinical histories which contained data regarding the race of the patients, it was found that 74 cases, or 60 per cent, were Caucasians and 38 cases, or 30 per cent, were negroes. Other races reported in the remaining 10 per cent included: the Mongolian (6 cases); the Malayan (2 cases); the Hindu (2 cases); a Mexican "mestizo" and a Jamaican.

A large majority of the victims of chromoblastomycosis have been farm laborers working barefooted in the fields at the time infection was contracted. In a study of 73 patients whose histories included the occupation, it was found that 60, or 82 per cent, were engaged in this type of work, while three others were farmers who were also engaged in other occupations (carpentry, mining, nursing). Among the remaining patients, there were three day laborers, two animal tenders, one cobbler, one merchant, one printer, one literary worker, and one reported as a "city dweller."

The specific causative fungi of chromoblastomycosis will be discussed under "Mycologic Aspects" (*q. v.*).

**Treatment:**— Chromoblastomycosis has been subjected to many different methods of treatment with various degrees of success. When the lesions are small and discrete, as is usual in incipient cases, it may be possible to completely eradicate the infection by surgical excision or electrocoagulation. On the contrary, when the infection is advanced and the lesions numerous, extensive and confluent, these measures would be impracticable and treatment becomes a more difficult problem, the method of attack depending on the individual circumstances.

In late, lower-limb infections the leg and foot are liable to be elephantiasic and heavy; secondary bacterial complications and ulceration are not uncommon and may be disturbing to the general health; the lesions are foul-smelling and repulsive in appearance; the patient becomes physically disabled and mentally demoralized. Under such circumstances surgical amputation and an artificial leg might solve the therapeutic problem, provided that the patient is otherwise healthy, not aged, and is willing to undergo the operation. This procedure would not only eliminate the physical and mental sufferings inherent to the disease but would bring the victim back to an active, more productive and happier life.

When the patient is old or unwilling to accept radical surgery, or when the infection affects a region, other than the leg and foot, not so liable to serious complications, treatment should be conducted on more conservative principles and should consist of both systemic and local measures. Internally, many chemotherapeutic agents—including arsenic, mercury, bismuth, gold, antimony, methylene blue, and the iodides of potassium and sodium—have been used to combat the infection. Among these, only the iodides would seem to be partially effective. They should be administered both by mouth and intravenously when possible, and the daily dose should be gradually raised to, and maintained at, the limit of tolerance over long periods. By this method of therapy it has often been possible to reduce the fibrosis and the size of the lesions, some of which may even disappear, promoting a general feeling of optimism in the patient. However, careful follow-up observations have not offered convincing evidence of the curative action of these compounds. Recent experiments would seem to indicate that sodium sulfamerazine possesses distinct inhibiting properties against *Fonsecaea Pedrosoi in vitro* (Keeney *et al.*, 1944, p. 393). On the basis of this knowledge the drug is now being administered to Puerto Rican patients, but it is too early yet to evaluate its true merits as a therapeutic agent in chromoblastomycosis.

Local treatment should aim, in the first place, at the elimination of all incipient efflorescences and as many of the older ones as can be effectively removed surgically or by electrocoagulation. Since the disease is slow in spreading and the possibilities of metastases are so meager, the fulfillment of that aim should check further progress of the infection. In the second place, lesions of larger size should be destroyed as far as possible by similar methods, and local treatment then conducted on general principles, giving preference to sodium sulfamerazine preparations. Iontophoresis with copper sulphate, which was employed with good results in a patient with lesions on the hand and forearm (MARTIN *et al.*, 1936) is worthy of further trial. Treatment with Roentgen rays has often promoted the improvement or complete clinical disappearance of some lesions. However, follow-up observations have not been satisfactory and recurrences on the radiated areas have been observed. This method of therapy might, perhaps, be effective on lesions of limited extent, but further investigative work is required for the development of an adequate technique.

## MYCOLOGIC ASPECTS

**Introduction:**— Chromoblastomycosis has been ascribed to several species of dematiaceous fungi, the biology of which is only partially known. These fungi may be classed in two different groups. The first group consists of organisms that are moldy in appearance, habitually producing an abundance of healthy, dry, aerial hyphae in laboratory cultures. In this group are included *Fonsecaea Pedrosoi* (BRUMPT, 1922) NEGRONI, 1936 *comb. nov.; Fonsecaea compactum* (CARRIÓN, 1935) CARRIÓN, 1940, *comb. nov.; Phialophora verrucosa,* MEDLAR, 1915; *Hormodendrum species* (SIMSON *et al.*, 1943); *Hormodendrum species* (O'DALY, 1943); and *Torula poikilospora,* TAKAHASHI, 1937. The etiologic importance of *Fonsecaea Pedrosoi, Fonsecaea compactum,* and *Phialophora verrucosa* has been generally accepted. The other three species mentioned are not so generally known, and some of them have not been extensively studied, but there are good reasons to suspect their possible etiologic relation to the disease, and it has been thought convenient to include them in this discussion.

The second group of fungi associated with chromoblastomycosis consists of four organisms which produce primarily soft, dark, moist colonies on the usual laboratory media, undergoing what appears to be a yeast-like phase in the course of their development. As the cultures age, a good part of the thallus becomes filamentous, but the growth as a whole retains its moist appearance. Only two of the organisms of this group have been published in the literature, and they were described as new species, namely, *Hormiscium dermatitidis,* KANO, 1937; and a "black *Candida*-like . . . species" (BERGER *et al.*, 1945). Of the two remaining fungi, one was isolated by BONNE from a patient with chromoblastomycosis in the East Indies,[2] and the other was obtained by us from a Puerto Rican case. The descriptions

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[2] A culture of the fungus isolated by BONNE was kindly given to us by Dr. CHESTER W. EMMONS, of the National Institute of Health at Bethesda, Maryland, U. S. A.

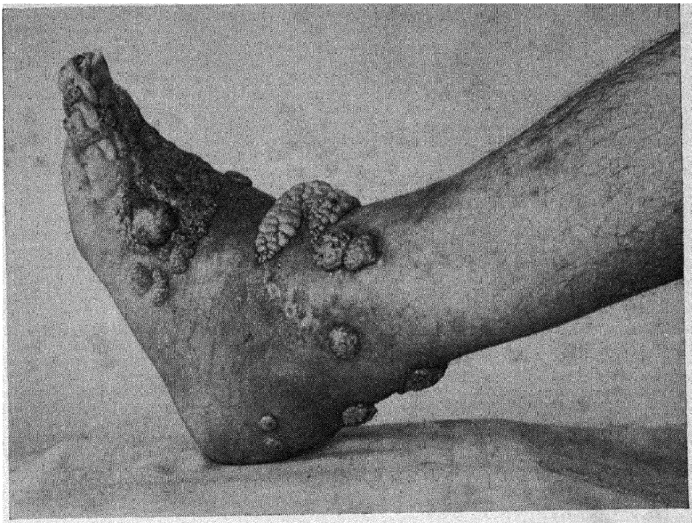

FIGURE 1. — A Puerto Rican case of chromoblastomycosis of fifteen years' duration. The lesions occurred in great numbers, especially toward the distal portion of the extremity, and they were conspicuously varied in morphology.

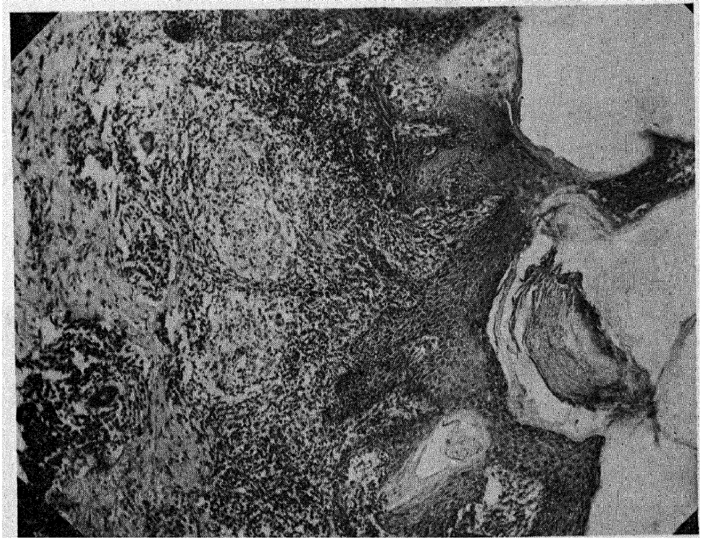

FIGURE 2. — Histopathologic section of a lesion in chromoblastomycosis. The epidermis is thickened and folded to fit the underlying papillomatous elevations. In the cutis the reaction is essentially granulomatous with a varied cellular infiltrate.

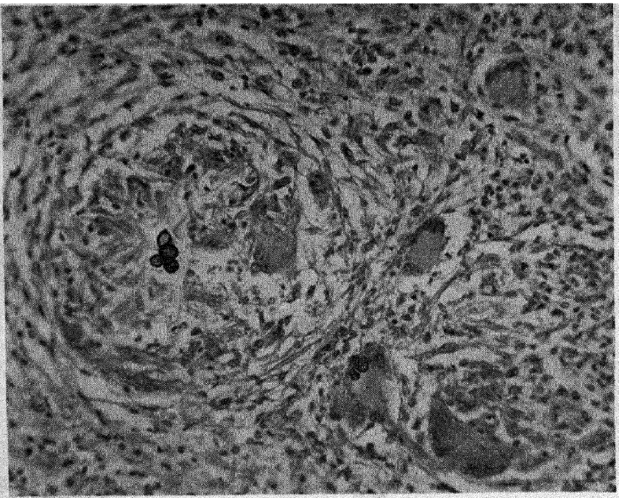

FIGURE 3. — Histopathologic section of a lesion in chromoblastomycosis showing a tuberculoid type of reaction. Note fungus elements in center of tubercle, and also within giant cell.

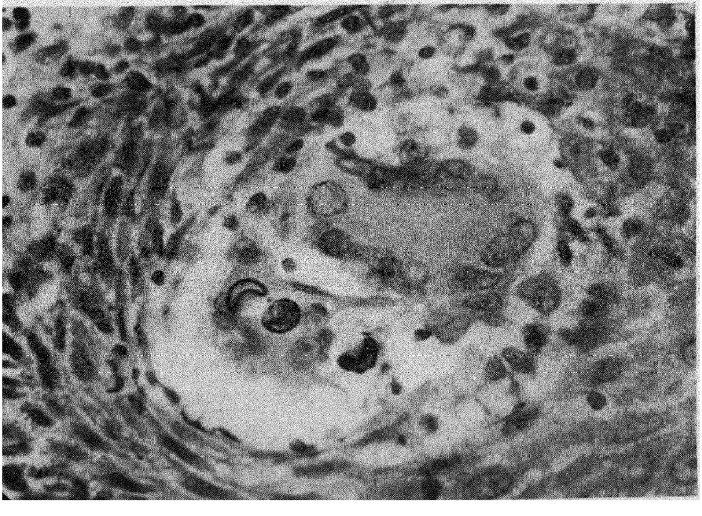

**FIGURE 4. — Histopathologic section of a lesion in chromoblastomycosis showing fungus in crescent-shaped form. Note also degenerated fungus element within giant cell.**

of these two fungi are not yet available. From a preliminary study of the four organisms included in this group, we have been led to suspect the existence of close interrelations among them. Further comparative studies are now being conducted in our laboratories with a view toward elucidating their true taxonomic position and relationship.

**Fonsecaea Pedrosoi**:—*Authorship.*—(Brumpt, 1922) Negroni, 1936 *comb. nov.*, Carrión, 1940 *emend.* — *Synonymy.* — *Hormodendrum Pedrosoi* (Brumpt, 1922); *Phialophora verrucosa* (Pedroso and Gomes, 1920); *Acrotheca Pedrosoi* (Fonseca and Leão, 1923); *Hormodendrum algeriensis* (Montpellier and Catanei, 1927); *Acrotheca Pedrosiana* (Bonne, 1928, quoted by Barros-Barreto, 1943); *Trichosporium Pedrosianum* (Ota, 1928); *Acrotheca verrucosa* (Tschernjawski, 1929); *Trichosporium Pedrosoi* (Langeron, 1929); *Hormodendrum rossicum*[3] (Meriin, 1930); *Cladosporium algeriensis* (Vuillemin, 1931, quoted by Barros-Barreto, 1943); *Gomphinaria Pedrosoi* (Dodge, 1935); *Hormodendroides Pedrosoi* (Moore and Almeida, 1936, p. 543); *Phialophora macrospora* (Moore and Almeida, 1936, p. 543); *Botrytoides monophora* (Moore and Almeida, 1936, p. 543); *Phialoconidiophora Guggenheimia* (Moore and Almeida, 1936, p. 543); *Hormodendrum japonicum*[3] (Takahashi, 1937, p. 53); *Hormodendrum Negroni*[4] (Pereira, 1938); *Carrionia Pedrosoi* (Briceño-Iragorri, 1938); *Hormodendrum chaquense*[4] (Mazza and Niño, 1939); *Phialophora Pedrosoi* (Binford *et al.*, 1944).

*Geographic distribution.* — *Fonsecaea Pedrosoi* has never been found in nature outside of the human body, but the widespread distribution of its victims would seem to indicate its ubiquity. *F. Pedrosoi* is by far the most common etiologic agent of chromoblastomycosis. Among 90 legitimately classified organisms isolated from the disease throughout the world 76, or 84 per cent, belong to this species. The geographic distribution of the fungus is conspicuously higher in the warmer climates. This is evident from the fact that 63, or 83 per cent, of the 76 isolates above mentioned, were obtained from patients who contracted the infection in tropical or subtropical regions, while only 13, or 17 per cent, were found in temperate zones. It might be of interest to note here that these proportions appear to be reversed in the case of another important etiologic species of chromoblastomycosis, namely, *Phialophora verrucosa,* of which there are 6 known isolates and 5 of them, or 83 per cent, correspond to the colder climates (see below: *"Phialophora verrucosa"*). However, the fact that most of the isolates of *P. verrucosa* have been found in temperate regions is no indication of predominance, for even in such regions the incidence of *F. Pedrosoi* has been found to be higher than that of *P. verrucosa* in a proportion of 13 to 5.

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[3] The fungus described under this name was not available for comparative studies in our laboratories, but the original description of the organism, together with the published illustrations, is strong evidence of its identity with *Fonsecaea Pedrosoi.*

[4] Authentic cultures of the so-called *Hormodendrum Negroni* and *Hormodendrum chaquense,* respectively, were kindly sent to us by Dr. Juan A. MacKinnon, of Uruguay. We agree with Azulay (1944) that these two fungi are identical with *Fonsecaea Pedrosoi.*

The continental distribution of *F. Pedrosoi* has been found to be as follows: North America, 5 isolates, including 4 from the United States and 1 from Mexico; Central America, 4 isolates, including 3 from the Canal Zone[5] and 1 from Guatemala; South America, 25 isolates, including 19 from Brazil, 4 from Venezuela and 2 from Argentina; West Indies, 33 isolates, including 18 from Cuba, 11 from Puerto Rico[6] and 2 from the Dominican Republic;[7] Europe, 4 isolates, all of them from Russia; Africa, 4 isolates, including 1 from Algiers and 3 from the Union of South Africa; Asia, 1 isolate from Japan; and the East Indies, 2 isolates, including 1 from Java and 1 from Sumatra.

*Morphology in pathologic tissue.* — Since chromoblastomycosis in human beings is essentially a skin disease, the infecting fungus is to be found almost exclusively in cutaneous lesions. Here it may be recognized in both the dermis and epidermis as characteristic, usually spherical, occasionally crescent-shaped, brownish-yellow bodies, measuring about 10 microns in diameter (Fig. 4).

These bodies may occur either singly or in clumps, rarely in short chains. They are found within giant cells, or free in the tissues, or in the center of microabscesses, or, finally, enclosed within epithelial pearls in the Malpighian and horny layers of the epidermis.

The fungus elements possess a dark, fairly thick cell wall, part of which is occasionally swollen, bulging toward the interior of the cell. The wall is sometimes covered with a crusty layer of refractile material. In histopathologic preparations stained with hematoxylin and eosin, this layer takes the eosin stain (TSCHERNJAWSKI, 1929; TIBIRIÇÁ, 1939; WEIDMAN and ROSENTHAL, 1941, and others), but it may be seen just as well in fresh unstained preparations of the infected epidermal scales (Fig. 5a). Not infrequently, the cell wall appears thin and degenerate due, undoubtedly, to the defense reaction of the body tissues. The protoplasm is granular and usually contains variously-sized, mostly coarse, spherical or irregular refractile inclusions, and it possesses a conspicuous natural pigment which has been described as ochre, olivaceous, yellowish-green, and chestnut brown. The nucleus is not apparent in fresh or stained preparations. Germination of the parasitic cells is frequently observed in the *stratum corneum* of the epidermis, the filaments being comparatively short, irregular, septate and branched, and showing the same pigmentation and structure noted in the mother elements (Fig. 5c). The authors have never seen typical filamentous structures in the dermis, but some investigators have reported their occurrence in this location (TERRA *et al.,* 1922; FONSECA and LEÃO, 1930, and others).

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[5] Of the three isolates from the Canal Zone, only one has appeared in the literature (SNOW *et al.*, 1945); the other two were studied in our laboratories due to the courtesy of Drs. E. S. WEDDING and C. CALERO, respectively.

[6] Only four of the Puerto Rican isolates have appeared in the literature (CARRIÓN and KOPPISCH, 1933; CARRIÓN, 1938).

[7] Of the two isolates from the Dominican Republic, only one has appeared in the literature as far as we know (CARRIÓN and PIMENTEL-IMBERT, 1938); the other was studied by the authors due to the courtesy of M. F. PIMENTEL-IMBERT.

In the infected tissue, multiplication of the fungus takes place by fission, different stages of the process being noticeable in both the dermis and epidermis. The first stage is represented by the development of an internal septum which divides the fungus cell into two hemispherical compartments (Fig. 5b). The parasitic body is thus transformed into a bicellular structure. Upon further growth, each of the two compartments tends to reproduce the spherical shape of the mother cell and a constriction becomes apparent at their plane of contact (Fig. 5b). Finally, the two daughter elements may separate from each other and become free cells, but they often remain united and, on maturity, they start again the process of fission. The repetition of cell division without separation and the fact that the dividing process may take place in more than one plane (Fig. 5c) often lead to the formation of irregular conglomerations of fungus cells (Fig. 3) not unlike the sclerotia sometimes noted in old fungus cultures. It is thus that the parasitic cells seen in tissues have been frequently described as "sclerotic cells."

*Fonsecaea Pedrosoi,* as present in the host tissues, has often been called a "black yeast," or a *"Blastomyces,"* or a yeast-like fungus," and some investigators have mentioned the production of conidia in the lesions by budding. Indeed, histopathologic sections showing spherical fungus cells with bud-like outgrowths have been occasionally observed. However, neither the descriptions, nor the illustrations published, nor the personal observations of the authors have yet offered convincing evidence that this fungus may multiply in the tissues by a budding process similar to that noted in North American blastomycosis which is produced by a different fungus, namely *Blastomyces dermatitidis.* The bud-like process noted in the infected tissues, might possibly represent germinating tips, or might result, perhaps, from a difference in the rate of growth of the two constituent elements of a septate fungus cell.

In animals experimentally infected, the parasite may be found in lesions produced, not only on the skin, but also in one or more of the internal organs, according to the route of inoculation. The morphology of the fungus in the pathologic tissues of such animals is essentially similar to that already noted in human lesions. Published descriptions mention the presence of spherical or oval, often septate, pigmented bodies, and also of filamentous structures, some of which are, undoubtedly, remnants of the inoculum.

In 1945, ARÊA-LEÃO *et al.* observed the presence of chestnut-colored "grains," of the type produced in maduromycosis, in the testicular lesions of a rat experimentally infected with the fungus of a Brazilian patient. The "grains" consisted of one or more sclerotic cells or mycelial filaments surrounded by numerous, closely set, but conspicuous, refractile, club-shaped bodies. Although the specific fungus was not named, it is presumable that these investigators were working with a specimen of *Fonsecaea Pedrosoi,* which is the parasite usually encountered in Brazilian chromoblastomycosis. In their report, they do not state, either, if the fungus was recovered in culture from the "grains."

*Gross morphology in culture.*[8] — On Sabouraud's *"milieu d'épreuve,"* after the fourth week and at room temperature, cultures resemble a flattened cone measuring about 5.5 cm. in diameter (Fig. 6) ; center of culture elevated about 8 mm. above medium, sometimes forming mammillary prominence; rest of culture gently sloping, often showing radial folding; border circular, or festooned; the aerial mycelium forming dark, gray, greenish, olivaceous-gray or dark-brownish, felt-like network of short, delicate, ascending hyphae arising from a more consistent, somewhat elastic, black, membranous mat; substrate mycelium light gray and moderately abundant. On 4 per cent dextrose agar, cultures usually less regular on the surface and in contour; aerial hyphae less intensely pigmented, more profuse and less uniformly developed. On Czapek's agar, colonies poorly developed; mycelium mostly submerged, gray to olivaceous, forming, at border, arborescent outgrowths; central zone showing shallow layer of aerial hyphae, consisting mostly of conidiophores.

*Microscopic morphology in culture.* — Microscopic characters usually more conspicuous after third week of growth. Vegetative hyphae long, straight or undulated, 1.25 to 3 microns in diameter, septate, branching, cell walls thick and dark; protoplasm olivaceous, granular, with refractile droplets.

Sporulation of three different types occasionally combined in same spore head: (*a*) *Hormodendrum* type (Fig. 7a) more or less abundant, generally more conspicuous on Czapek's agar cultures, sometimes predominating, sometimes obsolete, according to individual isolate; fertile branches erect or ascending, often arborescent, with terminal cell, or conidiophore, sometimes darker, having at tip several tiny, truncate, conical prominences to which spores are attached. Conidia borne in chains, usually short, which tend to branch by multiple budding at distal pole of each successive conidium, resulting in the formation of complicated spore heads (Fig. 7a); conidia unicellular, ovoid, often elongated, those at the base of the chain frequently shield-shaped and occasionally bicellular; measuring 3 to 5 by 1.5 to 3 microns (basal elements 2.5 to 3.5 by 7 to 10 microns); olivaceous in color, walls smooth, thick and dark; disjunctors moderately to poorly developed. (*b*) *Fonsecaea* type (Fig. 7b, c, d) more or less abundant, usually more conspicuous on corn meal agar cultures, sometimes predominating, sometimes obsolete according to individual isolate; conidiophores short or long, straight or irregular, exceptionally branched, consisting of one, sometimes more, articles disposed terminally, laterally, or intercalarily, often derived from spore element in *Hormodendrum* head; surface of conidiophore wholly or partly verrucous due to the presence of tiny, truncate, conical prominences to which spores are attached; pigmentation of conidiophore often darker than vegetative mycelium (Fig. 7b); conidia sometimes single (not catenate) forming clusters which may be indistinguishable, when terminal, from those of *Acrotheca* (Fig. 7b), some clusters showing tendency to chain formation as in *Hormodendrum* (Fig. 7d); morphology of conidia resembling terminal and subterminal elements of *Hormodendrum* heads already described. (*c*) *Phialophora* type (Fig. 7e and f), usually scant to moderate in abundance, rarely predominating; morphollogy similar to that of *"Phialophora verrucosa"* (*q. v.*).

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[8] The morphologic descriptions are given throughout this chapter in definition style for clearness and simplicity.

Chlamydospores of the "sclerotic cell" type already noted in tissue, sparsely produced in culture, the elements being spherical, 8 to 12 microns in diameter, with coarse, dark walls, protoplasm yellowish-brown, granular, usually containing variously sized, refractile inclusions; some elements showing cell division by internal septation in different planes, with production of bi-, tri-, or tetracellular structures.

*Morphologic varieties of Fonsecaea Pedrosoi.* — According to the various proportions in which the three methods of conidial formation above described may be represented in different isolates of *Pedrosoi,* this species has been subdivided into the following varieties:

1. *Fonsecaea Pedrosoi* var. *typicus* CARRIÓN, 1940, corresponding with *Pedrosoi* as originally presented by BRUMPT (1922); not a frequent variety; *Fonsecaea* sporulation most highly developed and in overwhelming predominance, especially on corn meal agar (Fig. 8a); *Hormodendrum* sporulation scant, or depauperate or abnormal in morphology; *Phialophora* sporulation usually rare.

2. *Fonsecaea Pedrosoi* var. *Cladosporioides* CARRIÓN, 1940, corresponding to fungi of the type described as *Hormodendrum algeriensis* (MONTPELLIER and CATANEI, 1927); *Hormodendrum* sporulation most highly developed and in overwhelming predominance; *Fonsecaea* sporulation scant; *Phialophora* sporulation usually rare (Fig. 9a, b, c).

3. *Fonsecaea Pedrosoi* var. *Phialophorica* CARRIÓN, 1942, represented by a single isolate described as *Phialophora macrospora*

(MOORE and ALMEIDA, 1936, p. 543); *Phialophora* sporulation in overwhelming predominance; *Fonsecaea* sporulation scant; *Hormodendrum* sporulation obsolete (Fig. 9d, e, f).

4. *Fonsecaea Pedrosoi* var. *communis* CARRIÓN, 1940, the most common variety encountered; sporulation of *Hormodendrum* and *Fonsecaea* types always abundant, although in various relative proportions; *Phialophora* sporulation sometimes as abundant as the other two types, but usually scant (Fig. 7).

In recent years, many isolates of *F. Pedrosoi* have been described as new and independent species on the basis of predominance of one or another of the methods of sporulation characteristic of this fungus and a great deal of time-consuming and painstaking work (CARRIÓN, 1940 and 1942) was required to establish the identity of such species with *Fonsecaea Pedrosoi.* The subdivision into varieties, as defined above, offers a broader concept of the species, it will promote adequate orientation for the classification of unknown isolates, and it will help to avoid the prevailing tendency to create new and illegitimate species.

*Behavior in artificial culture media.* — *Fonsecaea Pedrosoi* has been grown in a large variety of solid and liquid culture media. According to most investigators the formulae most appropriate for optimum development of gross cultural characters are those containing agar, 2 per cent, with 1 per cent of peptone, and 4 per cent of either maltose or dextrose as nutrients. In these media the fungus grows faster and more luxuriantly and the aerial mycelium is more abundant and forms more regular patterns, sometimes showing concentric zonation. These characters are specially noticeable in cultures produced on Sabouraud's *"milieu d'épreuve"* (SABOURAUD, 1910). The fungus has also been found to thrive luxuriantly on wort agar, tubers and root vegetables (potato, carrot, red beet, turnip, radish, gourd, manioc, sweet potato and others).

Cultures on the media above mentioned may be used, in addition, for the study of microscopic morphology. However, due to the fact that *Fonsecaea Pedrosoi* possesses three distinct types of sporulation, all of which are not always readily produced in such media, it is frequently necessary to resort to other formulae that are more stimulating to the process of sporulation in order to bring out the morphologic features of the fungus. Experience has shown that corn-meal infusion agar (white corn-meal, 4 per cent; agar, 2 per cent) and Czapek's synthetic medium as modified by Dox (1910), and by THOM and CHURCH (1926), not only stimulate the production of conidia, but each of them tends to induce more selectively a different type of sporulation. Thus, the *Hormodendrum* sporulation is more conspicuous, as a rule, on Czapek's agar; whereas the *Fonsecaea* type is usually better developed on corn-meal agar. The use of these two media, therefore, may be essentially important in the diagnosis of certain isolates of the species *F. Pedrosoi* and its varieties. It has been

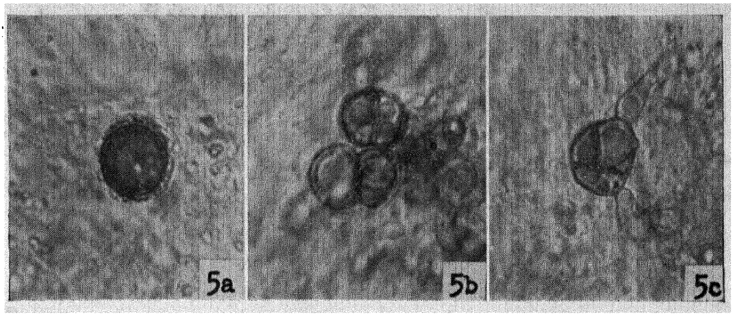

FIGURE 5. — *Fonsecaea Pedrosoi* in the infected epidermal scales: (a) isolated fungus cell covered with a crusty layer of refractile material; (b) fungus cells in different stages of division; (c) fungus cell showing internal septation in two planes, and also germination.

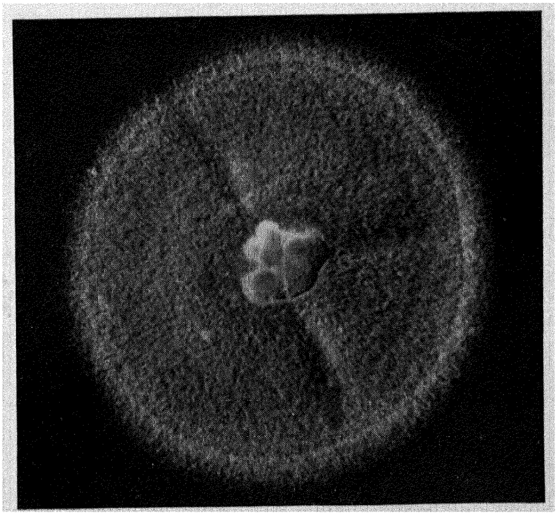

FIGURE 6. — *Fonsecaea Pedrosoi:* culture four weeks old developed at room temperature on SABOURAUD's "milieu d'épreuve".

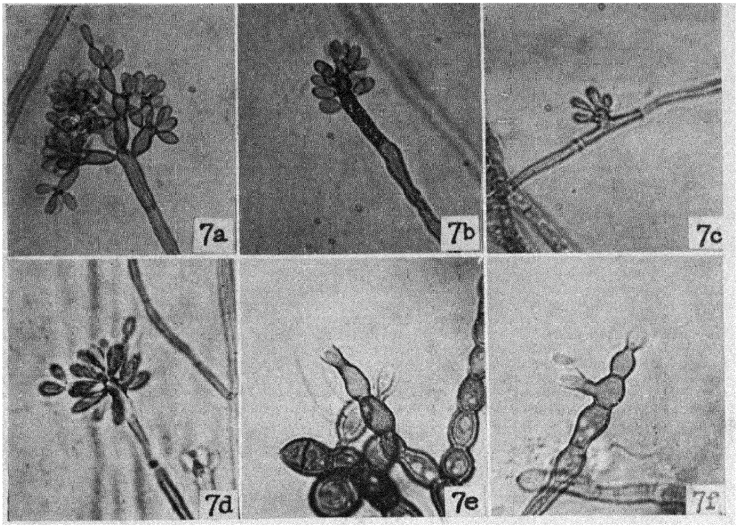

**Figure 7.** — *Fonsecaea Pedrosoi*, microscopic morphology: (a) *Hormodendrum* type of sporulation; (b) *Fonsecaea* type of sporulation, here resembling *Acrotheca*, with hyperpigmented conidiophore; (c) *Fonsecaea* type of sporulation illustrating the verrucous conidiophore described by Negroni; (d) *Fonsecaea* type of sporulation resembling *Acrotheca* but showing tendency to chain formation; (e) *Phialophora* type of sporulation represented by terminal phialide with conidium in cup; (f) *Phialophora* type of sporulation: note terminal and intercalary phialides.

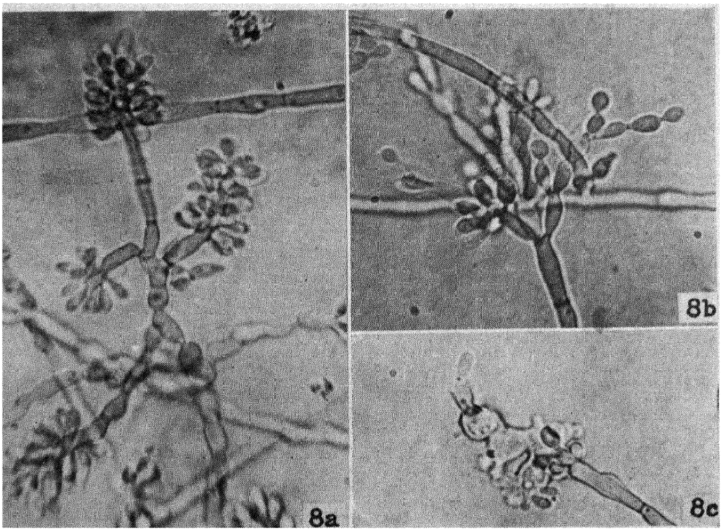

**Figure 8.** — *Fonsecaea Pedrosoi* var. *typicus* with: (a) the *Fonsecaea* method of sporulation predominating; (b) abnormal conidial chains in *Hormodendrum* head; (c) phialide with conidium.

claimed that the *Phialophora* method of sporulation becomes more evident on Czapek's agar (MOORE and ALMEIDA, 1936, p. 59). From personal experience we have been led to suspect that this claim may be correct, but further observations will be required before definite conclusions can be established on this point.

*Hydrogen-ion concentration, temperature, and oxygen requirements.* — It is generally admitted that *Fonsecaea Pedrosoi* requires an acid medium with a *p*H ranging between 5.5 and 6.5, and it has been claimed (BRICEÑO-IRAGORRI, 1938) that, when the *p*H is above 7, growth is delayed and may even be inhibited.

The optimum temperature for this fungus has not been precisely determined. However, its mycelium develops profusely at room temperature and some of the most important studies on its morphology have been conducted on cultures produced under ordinary laboratory conditions. It has been reported that artificial incubation at 37° or 37.5° C. promotes better growth (MONTPELLIER and CATANEI, 1927; TAKAHASHI, 1937, p. 53; DE ALMEIDA, 1939); but there has been no definite claim, so far, of any substantial advantage derived from cultivation at such temperatures, except, perhaps, that primary sowings with infected material may disclose positive results a few days earlier when artificially incubated (PEDROSO and GOMES, 1920; WEIDMAN and ROSENTHAL, 1941; SNOW *et al.,* 1945), a fact that might be of value in cases requiring a prompt diagnosis and which show no fungus elements in the epidermal scrapings.

*Fonsecaea Pedrosoi* grows best under aerobic conditions. This is evident from the abundance of healthy aerial mycelium and superficial sporulation usually produced under normal laboratory conditions on solid culture media. There is no doubt, however, that the fungus may grow to a certain extent at reduced oxygen tensions. Close examination of agar cultures will invariably show a certain amount of mycelium growing in the substrate, a feature which is particularly conspicuous on Czapek's synthetic medium. Anaerobic growth has also been observed in stab cultures on glucose agar (WEIDMAN and ROSENTHAL, 1941) and on various liquid media. It should be noted, however, that mycelium produced under such conditions is usually depauperate and devoid of sporulation.

*Chromatic affinities.* — Although many attempts have been made to determine the staining reactions of *Fonsecaea Pedrosoi,* no essential information has been obtained, by this means, about the internal structure of the fungus cells. TSCHERNJAWSKI (1929) has stated that the parasitic elements stain dark blue with azure II; dark brown with methyl green pyronine; green with polychrome blue, and dark violet with the Gram-Weigert stain. According to POZOJEWA (1930) the fungus would take a faint blue color with methylene blue. In preparations stained with Giemsa, its color has been described as "dark blue" (NAUCK, 1931) and "dark green-blue" (BINFORD *et al.,* 1944). The fungus structures have shown no apparent reaction to Gram's method of staining (POZOJEWA, 1930; NAUCK, 1931), and, with the Ziehl-Neelsen technique, they take on a darker and somewhat reddish color (NAUCK, 1931). Finally, WEIDMAN and ROSENTHAL (1941) have noted that osmic acid would intensify the dark pigmentation of the conidiophores produced in culture.

*Biochemical reactions.*—The biochemical activities of *Fonsecaea Pedrosoi* do not appear to be important. In 1930 MERIIN tested the fermentative action of a Russian isolate, named *Hormodendrum rossicum* (*Fonsecaea Pedrosoi*), against saccharose, mannitol, glycerol, dulcitol, galactose, maltose, dextrose, and starch, with negative results. His fungus liquefied gelatine but would not coagulate milk. TAKAHASHI (1937, p. 53) made similar tests with another isolate, the so-called *Hormodendrum japonicum,* using dextrose, galactose, mannose, levulose, arabinose, xylose, rhamnose, saccharose, maltose, lactose, raffinose, dextrin, inulin, and mannitol, also with negative results. In the following year, a Venezuelan specimen of *F. Pedrosoi* failed to ferment levulose, maltose, saccharose, galactose, inulin, raffinose, or glycerol under aerobic and anaerobic conditions (BRICEÑO-IRAGORRI, 1938), and, in the same year, PEREIRA (1938) published his observation on a Brazilian isolate (*Hormodendrum Negroni*), reporting no liquefaction of gelatin; coagulation and acid production on milk; and no fermentation of raffinose, xylose, mannitol, adonitol, saccharose, arabinose, dextrin, glycerol, inulin, galactose, levulose, glucose, lactose, or dulcitol. MAZZA and NIÑO (1939) have stated that an isolate of *F. Pedrosoi* from Argentina (the so-called *Hormodendrum chaquense*) liquefied gelatin slowly, peptonized milk with mild acidification of the medium, did not liquefy agar, showed no action on starch, liquefied coagulated serum with brownish discoloration of the medium, did not ferment glucose, and did not alter the *p*H of glucose-containing media. Finally, CASTRO-PALOMINO *et al.* (1941), were unable to produce fermentation of maltose or lactose with two Cuban isolates.

*Immunologic reactions: intracutaneous tests.*—MERIIN (1932 and 1938) claims to have induced specifically positive intracutaneous tests in patients with chromoblastomycosis using, as antigens, elaborately prepared culture extracts of *Fonsecaea Pedrosoi* (*Hormodendrum rossicum*) isolated from two different patients.

*Complement fixation and agglutination tests.*—It was also MERIIN (1930 and 1932), who first obtained a positive complement fixation reaction in this disease, but his results were found to be nonspecific.

BALIÑA *et al.* (1932) observed an intensely positive complement fixation test in a patient with chromoblastomycosis in Argentina. The antigen consisted of a mixture of cultures of *Fonsecaea Pedrosoi* (*Acrotheca Pedrosoi*) and *Phialophora verrucosa,* the tests being controlled with the sera of other patients not suffering from chromoblastomycosis.

In 1936 MARTIN *et al.*, reported on a series of strongly positive and specific complement fixation reactions with the serum of a patient infected with *Fonsecaea Pedrosoi* in North Carolina (U. S. A.). The tests were performed with 12 antigens,[9] each of them consisting of a culture extract from a different isolate of *Pedrosoi*, including the one from North Carolina. This experiment was controlled with two additional series of tests. In the first control series, 16 other fungi, some of them pathogenic, were used as antigens against the patient's serum. In the second control series, the serum of 28 presumptive syphilitics, four of them positive, were tested, respectively, against three isolates of *F. Pedrosoi*. All the controls were negative.

In 1937 CONANT and MARTIN immunized each of two groups of rabbits, respectively, against a different isolate of *Fonsecaea Pedrosoi* by intracutaneous inoculation of the animals with a suspension of the respective fungus. Subsequent immunologic studies carried out with the sera of these animals showed that complement fixing antibodies were present in both sera, and in high titer, for both of the *F. Pedrosoi* isolates used in the experiment. It was also shown that the serum of the immunized animals would induce positive cross reactions, in an equally high titer, against *Fonsecaea compactum*, and, in a lower titer, against *Phialophora verrucosa* (Uruguay) ; thus confirming, immunologically, the morphologic relationship previously established among the species *F. Pedrosoi*, *F. compactum* and *P. verrucosa* (CARRIÓN and EMMONS, 1935; EMMONS and CARRIÓN, 1936, and 1937).

A few attempts to agglutinate *Fonsecaea Pedrosoi* with the serum of patients suffering from chromoblastomycosis have been unsuccessful (MONTPELLIER and CATANEI, 1927; MERIIN, 1930).

*Response to antibiotic, chemical, and therapeutic agents.* — 1. *Alcohol.* The observations of WEIDMAN and ROSENTHAL (1941) and of BINFORD *et al.* (1944) would tend to establish that ethyl alcohol (95 per cent) is lethal to the fungus. From long experience in our laboratory, where parts of the specimens received for culture are routinely treated with ethyl alcohol (80 and 95 per cent) prior to inoculation, we have been led to believe that this chemical may actually kill the parasite, but its effects are conditioned by the factors of time and concentration. Further investigations are now in progress with the purpose of gaining more definite knowledge on this point.

2. *Formaldehyde.* WEIDMAN and ROSENTHAL (1941) have reported what appeared to be growth of the fungus in a mixture of equal parts of formaldehyde (10 per cent) and dextrose bouillon. It was

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[9] In this series of tests, the authors used, in addition, other antigens prepared with *Phialophora verrucosa* and *Fonsecaea compactum*. The results of the tests with these antigens will be discussed below (see "*Phialophora verrucosa*" and "*Fonsecaea compactum*").

not stated if the organism could be recovered in culture from this mixture.

3. *Sulfonamides.* In a series of carefully conducted experiments to determine the *in vitro* action of sulfonamides on pathogenic fungi, KEENEY *et al.* (1944, p. 393) were impressed by the inhibiting effects of sodium sulfamerazine against *Fonsecaea Pedrosoi.* On the basis of these experiments, the clinical trial of this drug in patients with chromoblastomycosis, both by mouth and locally on the skin lesions, should be encouraged. As already stated (see above: "Treatment"), some of our patients are now receiving treatment with this sulfonamide, but the period of observation has been too short to warrant conclusions as to final results.

4. *Fatty acids.* KEENEY *et al.* (1944, p. 377) have also studied the effects of fatty acids on the same group of fungi *in vitro,* and have found that certain salts of these acids, and particularly sodium caprate and sodium undecylenate, possess striking fungistatic and fungicidal action against *Fonsecaea Pedrosoi.* In view of the toxicity of these salts for the albino mouse, they do not recommend the internal administration of such salts in the treatment of the deep mycoses of man, until further animal experiments warrant their use. There is no reason, however, why these new drugs should not be tried locally on the cutaneous lesions of chromoblastomycosis.

5. *Penicillin.* The sensitivity of *Fonsecaea Pedrosoi* to penicillin has been tested by KEENEY *et al.* (1944, p. 410), as well as by FLEMING and QUEEN (1946), with negative results in both cases.

6. *Other therapeutic agents.* The effects of other chemical preparations used in the treatment of chromoblastomycosis have been already discussed (see above: "Treatment").

*Host range.* Man is the only well known natural host susceptible to infection with *Fonsecaea Pedrosoi.* It is possible that a spontaneous infection noted by CARINI (1910) in the lungs and kidneys of a batracian (*Leptodactylus pentadactylus*), was produced by this or a closely related fungus. RUDOLPH (1914) observed in Brazilian cattle another disease similar to human chromoblastomycosis, but no information is given regarding the true nature of the pathologic process.

Many investigators have endeavored to produce artificial infection with *Fonsecaea Pedrosoi.* TAKAHASHI (1937, p. 53) reported on the reproduction of typical lesions of chromoblastomycosis in the healthy skin of a patient who was suffering from the disease and also in a volunteer not previously infected, by inoculating a suspension of *Fonsecaea Pedrosoi* (*Hormodendrum japonicum*) isolated from his patient. The induced lesions were consistent histopathologically with chromoblastomycosis and the etiologic fungus could be recovered in culture.

Attempts to produce artificial infection have been carried out on eight different species of experimental animals, namely, rats (RUdolph, 1914; Pedroso and Gomes, 1920; Tschernjawski, 1929; Meriin, 1930; Carrión and Koppisch, 1933; Takahashi, 1937, p. 53; Pereira, 1938; Briceño-Iragorri, 1938; Mazza and Niño, 1939; O'Daly, 1943; and Azulay, 1945); mice (Montpellier and Catanei, 1927; Meriin, 1932; Takahashi, 1937, p. 53; and Levy and Black-Schaffer, 1945); rabbits (Pedroso and Gomes, 1920; Montpellier and Catanei, 1927; Gomes and Pessôa, 1929; Meriin, 1932; Carrión and Koppisch, 1933; Takahashi, 1937, p. 53; Pereira, 1938; and Mazza and Niño, 1939); dogs (Gomes and Pessôa, 1929); monkeys (Rudolph, 1914; Gomes and Pessôa, 1929; Carrión and Koppisch, 1933; and Simson *et al.,* 1943); guinea-pigs (Pedroso and Gomes, 1920; Meriin, 1932; Baliña *et al.,* 1932; Takahashi, 1937, p. 53; Briceño-Iragorri, 1938; Pereira, 1938; Mazza and Niño, 1939; Simson *et al.,* 1943; and Azulay, 1945); pigeons (Montpellier and Catanei, 1927); frogs (Almeida, 1934). Successful infections have been claimed in all but the last two species enumerated above. The animals most frequently used in the tests were the rat (11 investigators), the guinea-pig (9 investigators), the rabbit (8 investigators), the monkey (4 investigators), and the mouse (4 investigators); but the higher proportion of positives occurred in the rat and the mouse in the order given. In most of the induced infections the inoculum consisted of suspensions of the fungus grown on laboratory media. Only a few investigators used tissue emulsions and purulent exudate from lesions to infect their animals. The routes of inoculation have varied with different investigators and with the animals used. The intraperitoneal route was successfully employed in rats by Meriin (1930), Carrión and Koppisch (1933), Takahashi (1937, p. 53), and by Mazza and Niño (1939); and in mice, by Meriin (1932), Takahashi (1937, p. 53) and Levy and Black-Schaffer (1945). The subcutaneous route was used with positive results in rats by Tschernjawski (1929), Meriin (1930), Takahashi (1937, p. 53), and Pereira (1938); and, in mice, by Meriin (1932), and Takahashi (1937, p. 53). Intracutaneous inoculation has been effective in rats (O'Daly, 1943), and possibly in rabbits (Gomes and Pessôa, 1929, and Mazza and Niño, 1939). The intratesticular route was used by Mazza and Niño (1939) and, with remarkable success, by Azulay (1945) in both rats and guinea-pigs; and finally, Levy and Black-Schaffer (1945) produced systemic infections in mice by the intravenous route. According to the above observations it would seem that, among the different animals tested, the rat and the mouse are the species most susceptible to infection with *Fonsecaea Pedrosoi;* that infection may be effected by more than one route of inoculation; and, finally, that the intraperitoneal, the subcutaneous, and the intratesticular are the most adequate routes to produce infection.

Laboratory animals artificially infected with *Fonsecaea Pedrosoi* have not shown the chronic, slowly progressive, incurable, clinical manifestations inherent to the human disease, but the lesions produced have been sufficiently characteristic to confirm the pathogenic properties of the fungus. Infection in laboratory animals may be more or less limited in extent or it may be systemic, and even fatal, according to the method of inoculation. The lesions are nodular; variously sized, usually small; sometimes suppurating; single, few, or numerous; cutaneous or internal (liver, lungs, kidneys, peritoneum, testicle, etc.); when cutaneous, sometimes verrucous. The histopathology represents a granulomatous type of tissue reaction similar to that noted in human lesions, with the infecting parasite constituting a prominent part of the picture.

*Taxonomy.* Up to the present time the species *F. Pedrosoi* has shown no evidence of sexual reproduction and, consequently, it has its place among the Fungi Imperfecti. In view of the general morphology, sporulating habits, and dark color of the parasite, it has been included in the order *Moniliales,* family *Dematiaceae.* Finally, the simultaneous occurrence, in this organism, of three types of sporulation, which correspond to the genera *Hormodendrum, Phialophora* and *Acrotheca,* respectively, has led to the classification of the species in the new genus *Fonsecaea* (NEGRONI, 1936) CARRIÓN *emend.* (1940 and 1942). *Fonsecaea Pedrosoi* has been subdivided into the four varieties *typicus, Cladosporioides, Phialophorica,* and *communis,* as already defined (see "Morphologic Varieties").

The generic position of the *"Pedrosoi"* group has been a much debated subject. Differences of opinion have arisen mainly from (a) the triple sporulating ability of the fungus; (b) the outstanding and misleading predominance of one of the sporulating types in each of the varieties *typicus, Cladosporioides,* and *Phialophorica;* (c) failure to recognize the specific identity of these three varieties as established by the existence of intergrading forms (variety *communis*) which link them together and preclude any subdivision of the group beyond the ranks of varieties. Fortunately, many names under which different members of this species were described in the literature, have fallen into synonymy (see "Synonymy").

At the present time, some respectable authorities refer to *"Pedrosoi"* as a *Hormodendrum,* while others believe that the parasite should be included in the genus *Phialophora. Hormodendrum* was the name used by BRUMPT in his original description of the species, and therefore, has the advantage or priority. It is further supported by the fact that most members of the *"Pedrosoi"* group present the *Hormodendrum* sporulation as an outstanding morphologic feature. On the contrary, *Hormodendrum* is objectionable because, by definition, this genus includes fungi where conidia are produced only in branching chains, and would not admit the varieties *"Pedrosoi typicus"* and *"Pedrosoi Phialophorica,"* where the characteristic *Hormodendrum* chains are considerably reduced, abnormal or obsolete, while other methods of sporulation, representative of other genera (*Phialophora* or *Acrotheca*) constitute the outstanding morphologic features. The genus *Hormodendrum* could be emended to legitimately admit the species *Pedrosoi,* but this would introduce a serious source of confusion in the taxonomy of the group. Indeed, the history of *Pedrosoi* has clearly shown that objections to the generic name *Hormodendrum* have contributed more than any other cause to the long and confusing synonymy of the species. According to the International Rules of Botanical Nomenclature (Chapter I, Art. 4, in C. W. DODGE, 1935), and as a matter of principle, it is objectionable to "use . . . names which may cause error or ambiguity, or throw science into confusion."

THOM (1940) has proposed to include *Pedrosoi* in the genus *Phialophora,* based on his opinion that the strains of that species and those of *verrucosa* are too homogeneous to warrant classification in different genera. Our own observations and those of others would not lend support to THOM's opinion in this respect. The occurrence of phialides in *Pedrosoi* is truly indicative of some relationship with *Phialophora,* but there is no doubt whatsoever that, morphologically, the *Pedrosoi* group is infinitely closer to *Hormodendrum* than it is to *Phialophora.* There is *only one* isolate of *Pedrosoi,* the so-called *"macrospora"* (MOORE and ALMEIDA, 1936, p. 543), in which the *Phialophora* sporulation is a predominant character; in *all* the other isolates the *Hormodendrum* method, or the *Fonsecaea* method—which THOM (1940) himself has interpreted as a reduced form of *Hormodendrum*—constitute the predominating feature. Moreover, EMMONS and CARRIÓN (1936) have shown that the *Phialophora* conidiophores in *Fonsecaea Pedrosoi* may be produced by transformation of one or more *Hormodendrum* spores in an otherwise normal *Hormodendrum* spore head, indicating that the *Hormodendrum* sporulation is the primary biologic character of the species. If there were no other alternative but to place *Pedrosoi* in either *Phialophora* or *Hormodendrum,* the latter should be considered the more legitimate (*1*) because the morphology of *Pedrosoi* is *by nature* closer to that genus, and, (*2*) because the species was originally described under that generic name which, thus, has the right of priority.

The occurrence of certain homogeneal characters among species does not always imply actual cogeneric relationships. This fact has been repeatedly recognized. The morphology and parasitic habits of the Dermatophytes, for instance, point to close interrelations among the members of that group; yet they have shown sufficient differences to warrant their classification into different genera. "The rusts, to take another conspicuous example, are caused by many undoubtedly related fungi which have evolved along divergent lines and are classified in different genera" (EMMONS and CARRIÓN, 1936, p. 703). Finally, it is quite evident that the *Phialophora* type of conidiophore is not an exclusive character of the genus *Phialophora,* since similar morphologic structures are to be found also in other fungi, such as *Pleurage anserina* (B. O. Dodge, 1936), *Podospora curvula* (Satina, 1917, quoted by B. O. Dodge, 1936), *Cylindrocarpon radicicola* (Wollenweber and Appel, 1913, quoted by van Beyma, 1943), which are classed in widely separated generic groups.

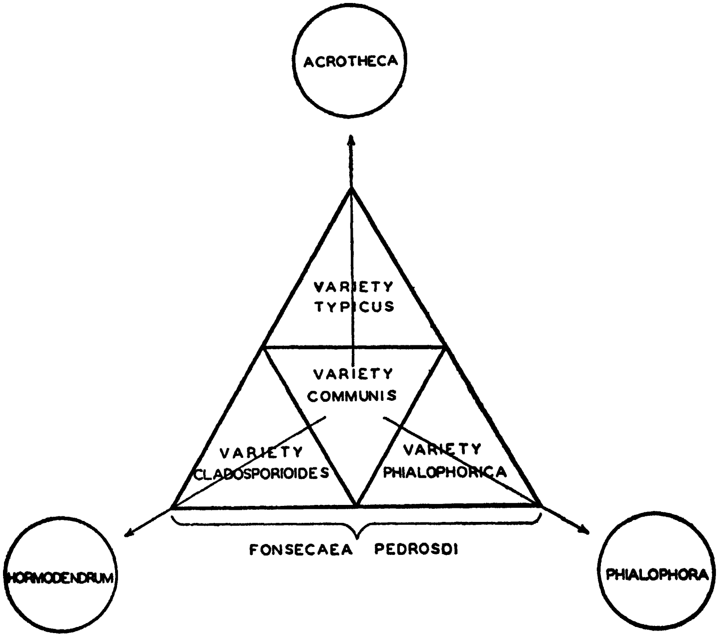

Figure 10. — *Diagram illustrating interrelations between the genus Fonsecaea and other related form genera.*—The large triangle represents a species of *Fonsecaea,* namely, *Pedrosoi.* The included smaller triangles are varieties of that species, and the circles represent the three genera to which *Pedrosoi* appears to be related, namely, *Hormodendrum* (*Cladosporium*), *Acrotheca,* and *Phialophora.* The arrows indicate suggested lines of evolution. *Fonsecaea Pedrosoi,* var. *communis* (center of triangle), possesses in conspicuous abundance the types of sporulation characteristic of the three genera above mentioned and, thus, would appear to represent their common origin. Different trends of evolution probably led to the development of the varieties *Cladosporioides, typicus,* and *Phialophorica,* each of which shows marked predominance of one of the types of sporulation, with equally marked reduction of the other two types. Further progress along the same line of evolution may have led to the differentiation of the three genera represented by circles, in which two of the sporulating types have disappeared completely, sporulation taking place, in each group, by a single method.

Binford *et al.* (1944) have accepted that a change in the generic name of *Pedrosoi* is necessary and, like Thom, they have stated their belief that this species should be transferred to the genus *Phialophora.* Their statement is made on the basis that "the *Phialophora* . . . sporulation was demonstrated *sparsely in several* strains of *Pedrosoi*" and *predominantly in one:*[10] the so-called *"macrospora"* (Moore and Almeida, 1936, p. 543). These observations are evidently in support of the fact that the *Phialophora* sporulation is not the most important morphologic character in *Pedrosoi* and, consequently, cannot be considered as a sound basis for transferring the species. It should be noted that the isolate called *"macrospora"* is not closer to *Phialophora* than the isolate called *"algeriensis"* is to *Hormodendrum.* Indeed, it is much easier to find sporulation of the *Acrotheca* type in *"macrospora"* than to find phialides in *"algeriensis."* If the generic position of *Pedrosoi* were to be solved by emending a preexisting genus, as suggested by Binford and his associates, the rule of priority would point, not to *Phialophora,* but to *Hormodendrum,* which is part of the original binomial used by Brumpt in his description of the species.

The classification of *Pedrosoi* as a *Fonsecaea* has been accepted on the following basis: (*1*) *Fonsecaea* (Negroni, 1936) Carrión, 1942, *emend.* is a legitimately created generic group including, at least two, well differentiated species, namely, *Pedrosoi* and *compactum;* (*2*) it admits, *a priori,* the simultaneous occurrence of the *Hormodendrum, Acrotheca,* and *Phialophora* methods of sporulation (Fig. 10) as noted in its constituent species; (*3*) it covers without strain the different varieties of *Pedrosoi;* (*4*) it leaves out fungi that are exclusively *Hormodendrum,* or *Acrotheca,* or *Phialophora* in type (Fig. 10); (*5*) it recognizes the existence of a characteristic *Fonsecaea* method of sporulation which is present in all the known strains of the group (Figs. 7, 8, 9), reaching its highest degree of development in *Fonsecaea Pedrosoi typicus* (Fig. 8); (*6*) as a generic name, it is neither misleading nor confusing, and, finally, (*7*) *Fonsecaea* has already become extensively popular, as noted in a review of recent publications on chromoblastomycosis.

In the preceding paragraphs we have presented the different trends of thought regarding the generic position of the species *Pedrosoi.* In view of the fact that this is an imperfect fungus possessing exceptional morphologic characters which link it closely to different form genera (Fig. 10), it seems probable that differences of opinion will continue to exist in the future. The authors believe, however, that, with the broad generic concept of *Fonsecaea,* and the subdivision of *F. Pedrosoi* into varieties, students in medical mycology will be able to work more effectively and lose less time in the classification of new or unknown isolates of this specific group. Further research toward determining the perfect phase of *F. Pedrosoi* in either its natural habitat—most probably the soil or vegetable matter therein—or in artificial media should be encouraged as the only way leading to a scientific solution of this taxonomic problem.

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[10] The italics has been added by us to the quotation.

**Phialophora verrucosa** MEDLAR, 1915:—*Synonymy.*—*Cadophora americana* (MELIN and NANNFELDT, 1934).

*Geographic distribution.*—*Phialophora verrucosa* has been encountered in only six chromoblastomycotic infections. The majority of these infections by *Phialophora verrucosa* occurred in continental United States and were distributed as follows: Boston, 1 case; Texas, 1 case; Missouri, 2 cases. The two remaining cases came from infections registered in Uruguay (South America) and Algiers (North Africa), respectively. Although the small number of *Phialophora* infections does not warrant final conclusions regarding the geographic distribution of the fungus as yet, it is of interest to note that five of the isolations were made in temperate climates while only one, the North African strain, corresponds to a tropical climate.

*Natural habitat.*—The isolation of a pathogenic fungus from its normal habitat in nature has been accomplished in only a few instances. In 1925, KRESS *et al.* (quoted by MARTIN, 1938), working with material from the wood pulp industry in the United States and Canada, reported the isolation of several dark colored fungi, some of which were later classified by MELIN and NANNFELDT (1934, quoted by MARTIN, 1938) as species of the genus *Cadophora* LAGERBERG, 1927. Subsequent studies have established beyond doubt that one of these species, the so-called *Cadophora americana,* is identical (CONANT, 1937; MARTIN, 1938) with *Phialophora verrucosa.* Thus, *Phialophora verrucosa* has become an additional member of the small group of pathogenic fungi so far discovered in their natural environment.

*Morphology in pathologic tissue.*—In parasitized cutaneous tissue the fungus is indistinguishable from *F. Pedrosoi* (Figs. 1, 2, 3). It may be observed in both the dermis and epidermis as characteristic, usually spherical, sometimes polyhedric bodies measuring 8 to 15 microns in diameter.[11] These bodies may occur singly or in clusters, rarely in chains. They are found within giant cells, free in the tissues, and in the center of microabscesses.

The fungus elements possess a dark and coarse cell wall, which may vary in thickness, even in an individual cell. The protoplasm is finely granular, yellowish-brown in color, and frequently contains variously-sized, mostly coarse, spherical or irregular refractile inclusions. There is no distinct nucleus discernible. Germination of the parasitic cells has been observed in old lesions or necrotic tissue, the filaments being short, thin-walled and septate, and showing the same pigmentation and structure noted in the mother elements (MOORE and MAPOTHER, 1940). In host tissues, the fungus evidently multiplies by internal septation, the process being identical with that already described in *Fonsecaea Pedrosoi* (*q. v.*). Multiplication by budding, endogenous sporulation and the production of conidia in tissue have

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[11] MONTPELLIER and CATANEI (1944) have observed, in host tissue, groups of sclerotic cells measuring four to six microns in diameter.

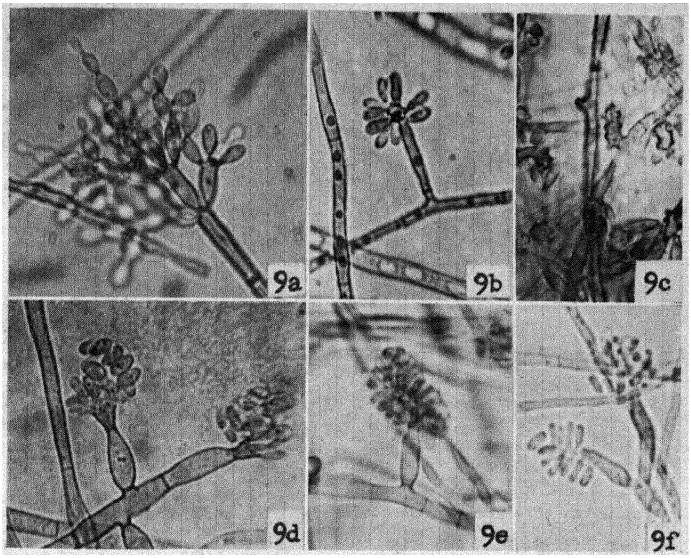

FIGURE 9. — *Fonsecaea Pedrosoi*, varieties *cladosporioides* (a, b, and c) and *phialophorica* (d, e, and f). Variety *cladosporioides* with: (a) the *Hormodendrum* sporulation predominating; (b) the *Fonsecaea* type of sporulation scant; and (c) verrucous conidiophore. Variety *phialophorica* with (d) phialides predominating; note the presence of *Fonsecaea* sporulation (e and f).

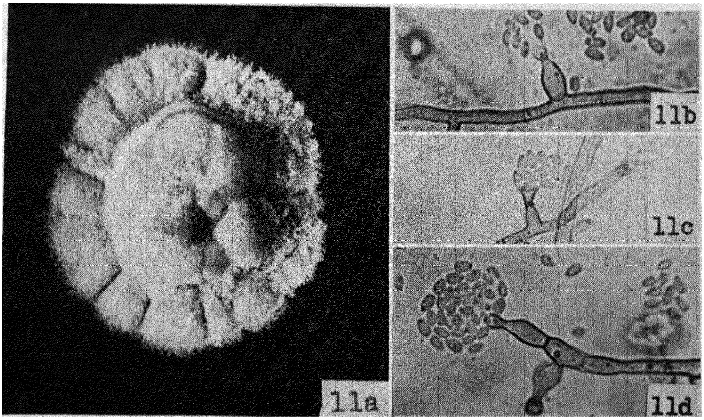

FIGURE 11. — *Phialophora verrucosa:* culture eight weeks old (a) developed at room temperature on SABOURAUD's "milieu d'épreuve". Note lateral and terminal phialides, and tendency of conidia to agglutinate at the opening of the conidiophore (b, c, and d).

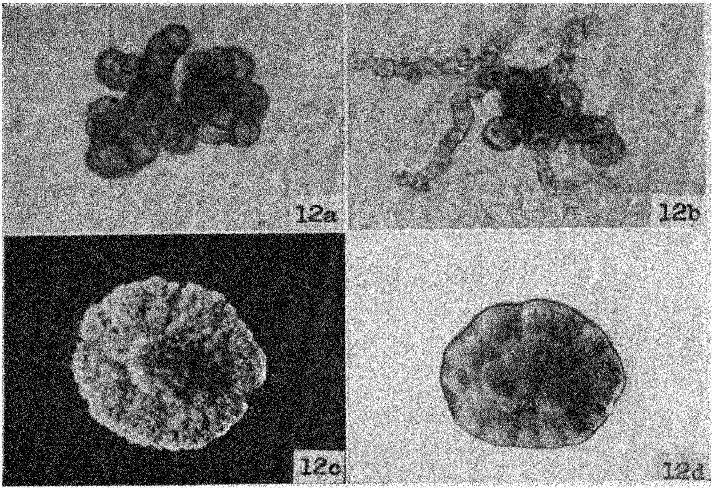

**FIGURE** 12. — *Fonsecaea compactum:* (a) aggregate of fungus cells resembling sclerotium in the infected epidermal scales; note internal septation in some of the cells; (b) germination of fungus cells in the infected epidermal scales; (c) culture six weeks old developed at room temperature on SABOURAUD'S "milieu d'épreuve"; (d) culture six weeks old developed at room temperature on 4 per cent glucose agar.

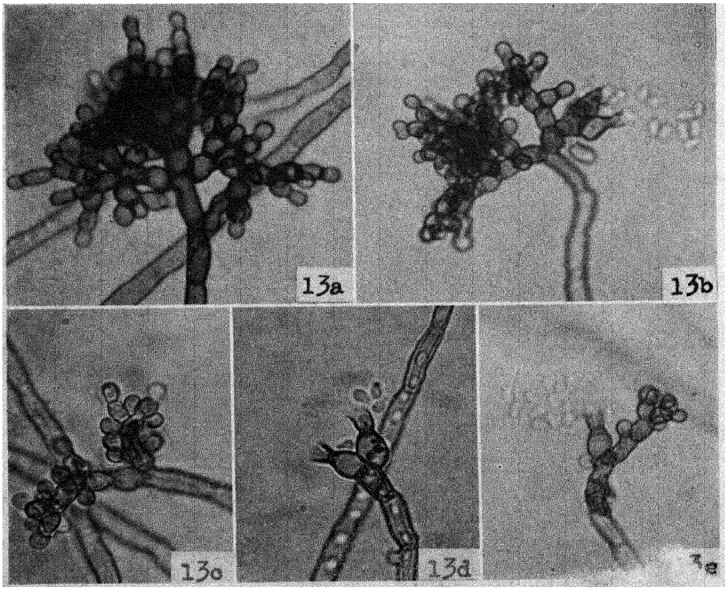

**FIGURE** 13. — *Fonsecaea compactum,* microscopic morphology: (a) *Hormodendrum* type of sporulation; (b) conidial head of *Hormodendrum* type with one of its conidia transformed into a sporulating phialide; (c) *Fonsecaea* type of sporulation; (d) *Phialophora* type of sporulation; (e) combination of the *Phialophora* and *Hormodendrum* types of sporulation produced terminally on same hyphal structure.

been variously reported in the literature. However, as in the case of *F. Pedrosoi,* neither the descriptions nor the illustrations published offer convincing evidence that *Phialophora verrucosa* may multiply in tissue by processes other than direct cell division.

In animals experimentally infected, the parasite may be found in lesions produced, not only on the skin, but also in one or more of the internal organs according to the route of inoculation. The morphology of the fungus in the pathologic tissues of such animals is essentially similar to that already noted in human lesions. Published descriptions mention the presence of spherical, often septate, pigmented bodies, and also of filamentous structures, some of which are, undoubtedly, remnants of the inoculum.

*Gross morphology in culture.* — Healthy colonies are produced on various laboratory media, but with great variation in the rate of growth according to individual isolates, cultures three weeks old on dextrose agar ranging in diameter between 0.5 cm. (Boston isolate) and 3.5 cm. (St. Louis isolate). Gross cultural characters generally more evident between the sixth and eighth weeks of growth (Fig. 11); cultures roughly conical in shape, measuring 3.5 to 6 cm. in diameter on the eighth week, with summit elevation of 5 to 10 mm.; surface irregular due sometimes to radial folding, sometimes to the development of extensive, prominent, lobular, central outgrowths, and sometimes to both types of alterations; border festooned or indented; aerial hyphae profuse, short, variously described as grey, dark olivaceous and dark brown, forming velvety or felt-like growth arising from a more membranous and darker mycelial mat which is attached to the substrate by a fairly thick growth of submerged hyphae.

*Microscopic morphology in culture.* — Microscopic characters usually more conspicuous between the fourth and sixth weeks of growth. Vegetative hyphae straight, cylindrical, branching, septate, with articles 8 to 25 microns long by 2 to 6 microns in diameter, septation often closer forming oidioid or moniliform mycelium; cell wall coarse, dark brown, with cell membrane thin and hyaline; protoplasm yellowish-brown, finely granular, and containing refractile droplets of various sizes; nucleus not apparent in fresh preparations, but visible with hematoxylin stain. Conidiophores either terminal or lateral, unicellular, measuring 5 to 12 by 2 to 3.8 microns, each conidiophore consisting of three parts (Fig. 11): the base, largest of all, containing protoplasm and nucleus; a constricted portion or neck; and a cup-shaped outlet ranging in diameter, at the lip, from 1.1 to 4.7 microns, usually from 2 to 3; as a rule, neck and cup forming the terminal portion of the conidiophore which thus becomes flask-shaped; otherwise cup and neck arising laterally from fertile hyphal article. Conidia semiendogenous, suggesting spermatia of Ascomycetes, produced singly and successively by budding through neck into cup, often numerously, overflowing cup and agglutinating to form spherical spore masses loosely adherent to cup; conidial elements oval, sometimes elongated, surface smooth, thin-walled, hyaline, measuring 0.8 to 2.8 by 1.4 to 7.8 microns, mostly 2 to 4 by 1 to 2.

Chlamydospores resembling "sclerotic cells" noted in tissue, sparsely produced in artificial culture media. Elements large, spherical; with coarse, dark walls; protoplasm yellowish-brown, granular, usually containing variously sized, refractile inclusions; some elements showing cell division by internal septation in different planes, with production of bi-, tri-, or tetracellular structures.

*Behavior in artificial culture media.* — *Phialophora verrucosa* will grow in most of the laboratory media usually employed in medical mycology, but the morphology of the cultures varies according to the composition of the substrate. In media containing carbohydrates, and particularly, in dextrose agar, the colonies develop faster and aerial mycelium is produced in greater abundance (MEDLAR, 1915; WILSON *et al.*, 1933; MACKINNON, 1936; MONTPELLIER and CATANEI, 1944). These media are useful, therefore, for the study of gross cultural characters. Czapek's synthetic medium appears to stimulate the development of mycelium in the substrate, partially inhibiting aerial growth (MACKINNON, 1936; MOORE and MAPOTHER, 1940). In the isolate studied by MOORE and MAPOTHER, the rate of growth was found to be faster in Czapek's agar. According to MEDLAR, Loeffler's blood serum and hydrocele agar would tend to produce moist colonies in which the aerial mycelium would lose its usual velvety appearance.

Some strains of *Phialophora verrucosa* have the property of producing a diffusible, dark, sepia-brown pigment, the formation of which appears to be related to the composition of the medium. The Boston isolate exhibits this property equally well on hydrocele agar and dextrose agar (MEDLAR, 1915); the Uruguayan (MACKINNON, 1936) and the St. Louis (MOORE and MAPOTHER, 1940) isolates produce the pigment on dextrose agar only; while the Texas isolate (WILSON *et al.*, 1933) failed to stain the agar in culture.

The influence of ingredients of the medium on the microscopic morphology of the fungus has been studied by various workers. MOORE and MAPOTHER (1940) noted that the vegetative hyphae of *Phialophora verrucosa,* when grown on Czapek's and malt extract agars, have a smaller diameter than those developed in media containing peptone. The production of moniliform hyphae has been reported to be more prominent on media containing sugar, but this type of mycelium has also been produced on other laboratory media. MEDLAR (1915) observed that hydrocele agar and Loeffler's blood serum would induce the formation of chlamydospores of sclerotic type, which were not produced in any of the other media used in his studies. MOORE *et al.* (1943) confirmed the occurrence of sclerotic cells on the last named medium. However, the production of these morphologic structures does not appear to be limited to the two media above mentioned. Sclerotic cells have been found on Czapek's agar (MACKINNON, 1936; EMMONS and CARRIÓN, 1936, p. 703) and it is possible that they can be produced on other substrates, though perhaps in more scant numbers.

According to some investigators, Czapek's agar would be the most adequate substrate to stimulate sporulation in this species. It has been stated that, in this medium, phialides are produced in greater numbers and in the largest variety of forms (MOORE and MAPOTHER, 1940; MOORE *et al.*, 1943).

*Hydrogen-ion concentration, temperature, and oxygen requirements.* — *Phialophora verrucosa* thrives best on an acid substrate. The hydrogen-ion concentration of the media used in cultural studies of this fungus has ranged between 4.3 and 7 (MOORE and MAPOTHER, 1940). Its optimum temperature has not been precisely determined, but the fungus is known to develop well at 37° C. (MEDLAR, 1915) and at 28° C. (MACKINNON, 1934). In our own laboratories, satisfactory cultures have been obtained at room temperature (24° to 33° C.). Although growth is usually more luxuriant under aerobic conditions, the development of mycelium and sporulation in the depth of the substrate (MEDLAR, 1915; MACKINNON, 1936), especially in Czapek's agar, indicates that the organism may grow to a certain extent at reduced oxygen tension.

*Chromatic affinities.* — The chromatic affinities of *Phialophora verrucosa* were studied by MEDLAR in 1915 and, since then, no essential addition has been made to his observations. In smears from cultures, the fungus elements would stain well, though diffusely, with all the routine bacteriologic stains, structural details being more evident after staining with dilute methylene blue for ten minutes or longer, followed by thorough washing in water. In fresh preparations of the mycelium stained with hematoxylin, practically all the cells reveal the presence of a nucleus and, in the case of conidia, the nucleus also becomes visible with eosine and methylene blue. The protoplasmic droplets noted in vegetative elements and in conidia, stain bright red with scharlach R and with carbol fuchsin, showing that they are fatty in nature. It has been found also that young fungus cells are Gram-positive, while the older structures take the blue irregularly when stained by Gram's method.

According to MEDLAR, culture sections fixed in Zenker's fluid should be stained with eosine and methylene blue, or with Mallory's iron hematoxylin, or with phosphotungstic acid hematoxylin, the latter stain being specially valuable for its decolorizing effect on the dark cell wall of the fungus, which permits more definition in structural detail. For culture sections fixed in alcohol, Mallory's hematoxylin or alum hematoxylin are equally effective. MEDLAR states further that the fungus in tissue may stain satisfactorily with the usual eosine-methylene-blue reagents.

*Biochemical reactions.* — Medlar reported the following biochemical reactions for *Phialophora verrucosa.* In litmus milk, no pellicle is formed, the milk is not coagulated or peptonized, and is gradually changed to a strongly alkaline reaction. In Dunham's peptone solution, no indol is produced, and the medium is colored a dark brown to chocolate brown in old cultures. The fungus does not ferment lactose, maltose, saccharose, mannitol, inulin, or levulose. Slight acid production occurs in dextrose and dextrin.

*Virulence.* — From the clinical picture noted in human infections, the slow course of the disease and the histopathologic reactions noted in the infected tissue, it is evident that *Phialophora verrucosa* is an organism of comparatively low virulence. In 1933 Wilson *et al.* made experimental inoculations to determine the variation in virulence produced by animal passage, noting that "Passage increased the virulence to a slight extent as judged by the greater emaciation of the animals and the somewhat larger size of the lesions."

Moore (1942) claims to have succeeded in producing infection of the chorio-allantois of chick embryos with the Boston and St. Louis strains of *Phialophora verrucosa.* The Boston strain, which had been kept in culture in the laboratory for 27 years, induced a definite, but mild and superficial reaction; while the St. Louis strain, of more recent isolation (four years), led to the development of more intensive and deeper lesions, indicating a higher degree of pathogenicity. As a result of his experiments, Moore was led to the conclusion that experimental infection of the chorio-allantois of chick embryos may be considered a more valuable and quicker method for determining comparative virulence among fungi, than the ordinary inoculation of other laboratory animals.

*Immunologic reactions.* — In 1936 Martin *et al.* demonstrated the presence of complement-fixing antibodies for *Phialophora verrucosa* in the serum of a patient infected with *Fonsecaea Pedrosoi.* In a subsequent series of well controlled experiments, Conant and Martin (1937) showed that the serum of a rabbit artificially immunized against *P. verrucosa* (Uruguayan strain) possessed complement-fixing antibodies, in high titer, for this species. The complement fixation test was also positive, although in a low titer, for *Fonsecaea Pedrosoi,* and it was negative for *Fonsecaea compactum.* It was further shown that anti-*Pedrosoi* rabbit serum, which produced an intensely positive reaction with a *Pedrosoi* antigen, would also react with *P. verrucosa,* although in a comparatively lower titer. Finally, anti-*compactum* rabbit serum was found to induce positive reactions of practically equal intensities, not only with *F. compactum,* but also with *F. Pedrosoi* and *P. verrucosa.* The cross reactions noted by these investigators would point to the existence of both specific and group fractions in the antigenic structures of these three fungi.

A saprophytic strain of *Phialophora verrucosa* (*Cadophora americana*) isolated from wood pulp by KRESS *et al.*[12] (1925, quoted by MARTIN, 1938), has been shown to be antigenically similar to other pathogenic strains of the species (MARTIN, 1938).

*Host range.* — Man is the only known natural host susceptible to infection with *Phialophora verrucosa.* Artificial infection has been attempted in four species of experimental animals, namely, the rat (MEDLAR, 1915; WILSON *et al.,* 1933; MACKINNON, 1934); the mouse (MEDLAR, 1915); the chick embryo (MOORE, 1942) and the guinea-pig (MEDLAR, 1915). Success has been claimed in all except the last named species. In every instance the inoculum consisted of a suspension of the fungus grown on laboratory media. The routes of inoculation varied with different investigators and with the animals used. In rats and mice, infection could be elicited by subcutaneous, intraperitoneal or intratesticular inoculations; in chick embryos, the inoculation and infection were effected on the chorio-allantoic membrane. Although the attempts to produce infection with *Phialophora verrucosa* in experimental animals have not been numerous, the results obtained appear to be generally consistent with those noted in similar experiments with *Fonsecaea Pedrosoi* (*q. v.*).

*Taxonomy.*— The species *P. verrucosa* has not shown a sexual phase of reproduction in laboratory cultures and should be classed, therefore, among the Fungi Imperfecti. It is remarkable that the semiendogenous spores produced by this species resemble the spermatia noted in certain types of Ascomycetes. However, attempts to induce ascus formation by the pairing of different strains in laboratory cultures have not been successful (N. F. CONANT, personal communication to the authors).

The production of conidiophores from superficial hyphae at any point on the surface of the thallus places this fungus in the third order of SACCARDO'S classification, namely, the *Hyphomycetales* (*Moniliales*); while the dark color of the cultures sets it among the *Dematiaceae.* Since the conidia are dark and unicellular, the position of the fungus lies in the first division of this family, with the *Phaeosporae.* According to the method of conidial formation, it falls in the subdivision *Chalareae.* The species was placed in the new genus *Phialophora* MEDLAR at the suggestion of THAXTER (MEDLAR, 1915) due to the agglutination of the conidia into sporangium-like masses at the mouths of the phialides. Finally, the specific name *verrucosa* was selected because the lesions produced by the fungus resemble those of *tuberculosis verrucosa cutis.*

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[12] MELIN and NANNFELDT (1934) classed this fungus as *Cadophora americana.* In 1937 CONANT established its identity with *Phialophora verrucosa.*

**Fonsecaea compactum**:—*Authorship.* — (Carrión) Carrión, 1940, *comb. nov.* *Synonymy.* — *Hormodendrum compactum* (Carrión, 1935); *Phialoconidiophora compactum* (Moore and Almeida, 1936, p. 543); *Phialophora compactum* (Binford *et al.,* 1944).

*Geographic distribution.* — Up to the present time, there are only two recognized representatives of the species *Fonsecaea compactum:* one of them is the fungus originally discovered in a case of chromoblastomycosis in Puerto Rico (Carrión, 1935); the other was isolated more recently from the skin lesions of a patient in the Armed Forces in the State of Tennessee (U. S. A.).[13]

*Morphology in pathologic tissue.* — The fungus is present in the cutaneous lesions and may be readily recognized in both the dermis and epidermis. It is represented by characteristic, usually spherical, sometimes hemispherical or ovoid, occasionally crescent-shaped bodies, measuring from 7 to 10.5 microns in diameter. These bodies occur either singly or in clumps. They may be found within giant cells, free in the tissue, in the center of microabscesses, and in the Malpighian and horny layers of the epidermis.

The fungus elements possess a dark and fairly thick cell wall. The protoplasm is granular, it is naturally colored yellowish-brown to olivaceous, and contains, as a rule, variously sized, mostly coarse, spherical or irregular, refractile inclusions. The nucleus is not apparent either in fresh or stained preparations. Germination of the parasitic cells is frequently observed in the *stratum corneum* of the epidermis (Fig. 12*b*), the filaments being comparatively short, irregular, septate and branched, and showing the same pigmentation and structure noted in the mother elements. Filamentous structures have not been observed in the dermis. In the infected tissue, multiplication of the fungus takes place by internal septation (Fig. 12*a*), the process being identical to that already described in *Fonsecaca Pedrosoi* (*q. v.*).

*Gross morphology in culture.* — On Sabouraud's *"milieu d'épreuve,"* growth slow; cultures six weeks old developed at room temperature, roughly conical in shape (Fig. 12*c*), measuring 2.5 cm. in diameter, with summit elevation of approximately 6 mm.; center of culture forming irregular mammillary prominence, about 10 mm. wide; rest of culture moderately sloping and uneven; marginal zone shallow, slightly depressed in places, showing faint tendency to radial furrowing; border irregular and indented; aerial hyphae profuse and plushlike, coarse, erect, brownish-black, arranged in characteristic tufts, arising from a more compact and brittle mycelial mat which is attached to substrate by a thin growth of submerged hyphae.

On 4 per cent dextrose agar, cultures essentially similar to the above (Fig. 12 *d*), but not as plushlike or bushy; aerial growth smoother and more velvety in appearance; hyphae finer; peripheral radial furrowing more pronounced; substrate mycelium extending slightly beyond border of culture, forming marginal rim, less than 1 mm. wide.

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[13] This isolate was kindly sent to our laboratory by Dr. Norman F. Conant, of Duke University, North Carolina, U. S. A.

On Czapek's solution agar, cultures characteristic, though poorly developed, measuring 10 to 16 mm. at the end of sixth week; mycelial growth chiefly in substrate, forming olive-black layer limited by an irregular, finely dentate border; center of colony showing scant aerial growth, powdery and dusty in appearance, brownish-black in color, reaching a thickness of about 1 mm. at point of inoculation.

*Microscopic morphology in culture.* — Microscopic characters usually more conspicuous after third week of growth. Vegetative hyphae long; coarse (2.5 to 5.2 microns); septate; branching, sometimes dichotomously; borders usually irregular; cell walls thick and dark; protoplasm olivaceous, granular, with refractile droplets; hyphal fusions frequent; nucleus not apparent in fresh preparations. Sporulation of three different types, occasionally combined in same spore head: (*a*) *Hormodendrum* type predominating (Fig. 13*a, b, e*). Fertile branches erect or ascending; often arborescent; with terminal cell (conidiophore) swollen, often flask-shaped and darker, having at tip broad facets to which spores are united. Conidia produced in short and profusely branching chains, some of the branches growing basipetally; conidial elements unicellular, spherical to subspherical, mostly barrel-shaped; cell wall smooth, coarse and dark; protoplasm olivaceous and granular; disjunctors absent, each conidium being compactly linked to its immediate neighbors in the chain by broad articulating facets; size of conidia 2.5 to 4.8 by 2.5 to 3.8 microns, basal elements 3.8 to 6 by 3 to 4.5 microns. (*b*) *Fonsecaea* type not conspicuously abundant (Fig. 13*c*). Conidiophores consisting of an article, usually short, straight or irregular; more darkly pigmented than vegetative mycelium; disposed terminally, laterally or intercalarily; sometimes produced by transformation of spore element in *Hormodendrum* spore head; surface of conidiophore irregular due to presence of broad facets to which spores are attached. Conidia individually similar in morphology to terminal and subterminal elements of *Hormodendrum* chains already described in type (a), but produced acropleurogenously, and singly (not catenate), forming clusters; some clusters, however, showing tendency to chain formation as in *Hormodendrum.* (*c*) *Phialophora* type (Fig. 13 *b, d* and *e*) scant, morphology similar to that of *Phialophora verrucosa* (*q. v.*).

Chlamydospores resembling "sclerotic cells" noted in tissue, sparsely produced in culture. Elements spherical, 8 to 12 microns in diameter; with coarse dark walls; protoplasm yellowish-brown, granular, usually containing variously sized, refractile inclusions; some elements showing cell division by internal septation in different planes with production of bi-, tri-, or tetracellular structures.

*Behavior in artificial culture media.* — *Fonsecaea compactum* will grow in most of the laboratory media usually employed in medical mycology, but the morphology of the cultures varies according to the composition of the substrate. The formula most appropriate for optimum development of gross cultural characters is one containing agar, 2 per cent, with 1 per cent of peptone and 4 per cent of either maltose or dextrose as nutrients. In media prepared according to this general formula, and particularly in Sabouraud's *"milieu d'épreuve"* (SABOURAUD, 1910), the fungus grows more luxuriantly, the aerial mycelium is produced in greater abundance, and the colonies form more regular patterns. The above mentioned media may be useful also for the study of microscopic morphology. However, corn meal agar and Czapek's solution agar appear to be more stimulating to the process of sporulation and are generally employed in morphologic studies of the fungus.

*Hydrogen-ion concentration, temperature, and oxygen requirements.*—*Fonsecaea compactum* requires an acid medium with a *p*H ranging between 5.5 and 6.5. Its optimum temperature has not been precisely determined, but the fungus develops well at both room (25° C.) and incubator (37° C.) temperatures. Although growth is usually more abundant under aerobic conditions, the development of mycelia in the depth of the agar, especially in Czapek's medium, indicates that this organism may grow at reduced oxygen tensions. It should be noted, however, that mycelia produced under such conditions are usually depauperate and devoid of sporulation.

*Immunologic reactions.* — In 1936 MARTIN *et al.* were able to demonstrate the presence of complement-fixing antibodies for *Fonsecaea compactum* in the serum of a patient infected with *Fonsecaea Pedrosoi.* In a subsequent series of well controlled experiments, CONANT and MARTIN (1937) showed that the serum of a rabbit artificially immunized against *F. compactum* possessed complement-fixing antibodies, in high titer, not only for the immunizing species, but also for *Fonsecaea Pedrosoi.* Conversely, the serum of another rabbit immunized against *F. Pedrosoi* produced equally strong positive reactions with culture antigens of both *Pedrosoi* and *compactum.* These observations are indicative of the existence of a close antigenic relationship between the two *Fonsecaeas: Pedrosoi* and *compactum.* The immune sera of the two animals also induced positive reactions, although in a lower titer, with a culture antigen prepared with *Phialophora verrucosa.* Finally, *Fonsecaea compactum* has been found to be antigenically related (MARTIN, 1938) to the so-called *"Cadophora americana"* (MELIN and NANNFELDT, 1934), a saprophytic strain of *Phialophora verrucosa* isolated from wood pulp.

*Viability.* — In a desiccated glucose agar culture of *Fonsecaea compactum* which was kept in the laboratory under ordinary conditions, the fungus was found to be still viable after a period of over two years. At the end of this period, inoculations of material from the dry culture into fresh media gave rise to characteristic cultures in which the specific morphology was typically reproduced.

*Taxonomy.* — The species *compactum* has failed to reveal a sexual phase of reproduction in laboratory cultures, and should be classed, at least for the present, among the Fungi Imperfecti. The production of conidiophores from superficial hyphae at any point on the surface of the thallus places it in the third order of SACCARDO's classification, namely, the *Hyphomycetales* (*Moniliales*). Its familial position, as determined by the dark color of the mycelium and conidia, is among the *Dematiaceae.* It has been included in the genus *Fonsecaea* because it possesses the three types of sporulation characteristic of that genus, namely, the *Hormodendrum,* the *Fonsecaea,* and the *Phialophora.* The specific name *compactum* was given in consideration of the compact articulation of the spores in the *Hormodendrum* chains.

**Hormodendrum Species:**— *Source.*—This fungus has been isolated from chromoblastomycotic lesions in only one instance, the infection taking place in a patient from the Cape Province, Union of South Africa. Original descriptions of the clinical picture in this patient and of the infecting fungus were published by SIMSON[14] *et al.* in 1943.

*Morphology in pathologic tissue.* — In the infected tissues the parasite was described (SIMSON *et al.,* 1943) as: "Brownish, round fungus bodies . . . lying free among the pus cells in the centres of the follicles, or included within the cytoplasm of the giant cells. These bodies measured 6 to 8 microns in diameter."

*Gross morphology in culture.* — The fungus grows very slowly, cultures five weeks old incubated at room temperature on 2 per cent glucose agar measuring only 2 cm. in diameter. "The surface is rather irregular in cross section, and, when the organism was first isolated, aerial hyphae" were not very abundant. "On repeated subculture, they increased in number until the surface became plushlike in character."

*Microscopic morphology in culture.* — Vegetative hyphae long, septate, branching, measuring about 2.5 microns in diameter. Sporulation of *Hormodendrum* type only: spores adjacent to conidiophore measuring 8 to 10 by 2.5 to 4 microns, their size gradually decreasing toward the distal end of the chain, "average intermediate spore being about 3-5 microns."

*Animal inoculations.* — "Heavy suspensions of the spores and hyphae of" this fungus "were injected subcutaneously into guinea-pigs and a *rhesus* monkey. Although the animals were examined repeatedly over a period of more than six months, no lesion developed."

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[14] In a recent publication, SIMSON (1946) reports the isolation of this species from two additional cases of chromoblastomycosis.

**Hormodendrum Species:**—*Source.* — This *Hormodendrum* was obtained from one of the lesions of a patient with chromoblastomycosis in Venezuela, the fungus being cultured from exudate aseptically aspirated from the lesion. Descriptions of the clinical picture and of the infecting parasite were published by O'Daly in 1943.

*Morphology in pathologic tissue.* — In purulent exudate from the cutaneous nodules the parasite is described as double-contoured, dark colored, sometimes septate bodies, occurring either singly or in clusters. These bodies are also represented in photomicrographs of the histopathology.

*Gross morphology in culture.* — Cultures six weeks old developed at room temperature on 4 per cent dextrose agar are irregularly circular in contour, and are superficially uneven. They measure 35 to 40 mm. in diameter, and, centrally, they are elevated about 6 mm. above the surface of the medium. The central portion of the culture is dome shaped, and is surrounded by a gently sloping, variously folded intermediate zone which is, in turn, surrounded by a smooth peripheral zone somewhat depressed below the surface of the medium. The border is festooned. Superficially the colonies are covered with aerial mycelium. In the center this mycelium forms a brownish-green, velvety network of short, delicate, ascending hyphae; over the rest of the colony the growth is finer and more powdery. The cultures are attached to the agar by a moderate growth of substrate hyphae which extend peripherally beyond the border of the aerial mycelium, forming a glistening-black marginal rim.

On 2 per cent dextrose agar the colonies are generally more filamentous, and, on honey agar, the central prominence appears more elevated and is covered with shorter, finer, and more glossy aerial mycelium.

At the time of isolation, the cultures produced a dark diffusible pigment, but this property was not apparent in subcultures.

*Microscopic morphology in culture.* — Microscopically this fungus has the general morphology of a *Hormodendrum.* The fungus has a fine mycelium, the spore chains are fairly long, and the spores are uniform in size. No further details are given in the original description.[15]

*Experimental inoculations.*—By inoculating spores of this *Hormodendrum* on the tail of white rats, O'Daly claims to have produced lesions indistinguishable from those produced by other etiologic fungi of chromoblastomycosis in the same animals.

**Torula poikilospora:**—*Source.*—This fungus was repeatedly isolated from purulent exudate and fragments of lesions in a case of chromoblastomycosis observed in Tokio, Japan. A report of the case and a description of the fungus were published by TAKAHASHI in 1937 (p. 31).[16]

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[15] A culture of this fungus was kindly sent to the authors for study by Dr. O'Daly. As far as we have observed, this is a good species of *Hormodendrum.* Comparative studies of this and other similar isolates obtained from chromoblastomycosis are being conducted in our laboratory.

*Morphology in pathologic tissue.* — The parasite, as observed in the exudate, consisted of "round or oval, double-contoured, light to dark brown bodies, mostly unicellular, seldom bicellular, measuring 5.5 to 9 microns in diameter." In tissue sections these bodies usually occurred "within microabscesses and giant cells, seldom in the lumen of newly formed blood vessels." In shape and color, they were similar to those noted in the exudate, but their diameter was given as 5.5 to 10 microns for the round forms, and 6-9 by 5.5-7.5 microns for the oval forms. The cell wall consisted of a coarse, dark brown, outer layer, and a thinner, light brown, inner membrane.

Lesions induced on experimental animals revealed the fungus structures in two different forms: the "yeast-like" and the mycelial. The "yeast-like" form corresponded closely with the round or oval bodies already noted in human lesions. The mycelial form could be best observed in the necrotic tissue of mouse lesions, and was represented by a network of olive-green, sparsely branched hyphae, 1.5 to 3.5 microns in width, divided into rather short articles by transverse septa. According to TAKAHASHI, the mycelial structures were less abundantly produced in rabbit and rat lesions, and they were never observed in guinea-pig infections.

*Gross morphology in culture.* — On Sabouraud's glucose agar, growth slow, colonies one month old measuring 3 to 3.5 cm. in diameter; shape of culture roughly conical, showing central hemispherical prominence, surrounded by a more extensive, gently sloping, radially furrowed zone; border circular, filamentous, finely arborescent; aerial hyphae short, downy, brownish-gray, arising from a soot-black, moist, granular mycelial mat.

On Sabouraud's maltose agar and on "Pollacci's medium," cultures essentially similar to the above. On plain agar and ascitic agar, growth slower, and furrowing less conspicuous. On potato and carrot plugs, growth slow, colonies hemispherical, reaching a diameter of 2 to 2.5 cm. after two months; covered by brownish-gray, short, and downy mycelium. On liquid media (glucose broth, maltose broth, and "potato water"), growth noted at the surface and bottom, the liquid remaining clear.

*Microscopic morphology in culture.* — Vegetative hyphae straight, undulated, or bent at acute angles; cylindrical; profusely branched, some filaments showing multiple terminal branching; septate, with articles 3 to 25 microns long by 1.5 to 4.5 microns wide; protoplasm olive-green, older hyphae more deeply colored and containing variously shaped deposits of a black pigment. Fertile hyphae undifferentiated. Arthrospores developed at tip and sides of filaments, forming long, branching chains, the acrogenous chains being longer and more branched than the pleurogenous; spores firmly linked to immediate neighbors in chain by broad articulations; spores olive-green, the older containing black pigment deposits; some spores spherical (2 to 9 microns in diameter) or oval (2.5 to 14 by 1.4 to 8 microns); most spores, however, irregular in shape, measuring 2 to 2.5 by 1.5 to 16 microns, the irregularity resulting from development of knob-like or short filiform appendages on the tip or on the side, never on the base, of spore cell, the appendages sometimes producing a secondary element and often separated from each other and from mother spore by septation.

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[16] Up to the present time, the authors have been unable to obtain a culture of *Torula poikilospora* for their collection. The description here presented has been abstracted from the work of TAKAHASHI.

Chlamydospores developed in old cultures, sometimes in chains; elements double-contoured, light brown to brownish-black; terminal or intercalary.

*Temperature requirements.* — The optimum growth temperature for *Torula poikilospora* is given as 37° C.

*Biochemical reactions.* — The fungus does not ferment glucose, galactose, mannose, levulose, arabinose, xylose, rhamnose, saccharose, maltose, lactose, raffinose, dextrin, inulin and mannite.

*Experimental infections.* — TAKAHASHI has reported the reproduction of typical lesions of chromoblastomycosis on the healthy skin of his patient, following local inoculations with suspensions of *Torula poikilospora.* The histopathology of the induced lesions was similar to that of the natural foci of infection noted in the clinical picture, and the etiologic fungus could be recovered in culture.

Experimental infections were also produced in rats, mice, rabbits, and guinea-pigs, susceptibility being more pronounced on the first three of the species mentioned. The inoculum used in these experiments consisted of saline suspensions of the fungus grown for three months on Sabouraud's glucose agar. The inoculations were made by the subcutaneous route in 4 mice and 2 white rats; the intraperitoneal route was used in 12 mice, 7 white rats, and 4 guinea-pigs; and the intravenous route, in 3 rabbits.

The subcutaneous inoculations were followed by the development of ulcerating nodules locally. The intraperitoneal inoculations produced progressive granulomatous nodules on all the abdominal organs of the rats and mice, and on the omentum of the guinea-pigs; finally, the intravenous inoculations induced a granulomatous reaction in the lungs of all the rabbits tested. Microscopically, the lesions were of granulomatous type and resembled those noted in human beings, with the infecting parasite constituting a prominent part of the picture.

*Taxonomy.* — *Torula poikilospora* is an imperfect fungus corresponding to the family *Dematiaceae.* Following the classification of SACCARDO-LINDAU, TAKAHASHI has included this species among the

*Toruleae*, and has placed it provisionally in the genus *Torula* PERSOON. The specific name *poikilospora* was given in accordance with the polymorphic character of the spores produced by the fungus.

**Hormiscium dermatitidis**:— *Source.—Hormiscium dermatitidis* was originally isolated from the lesions of a patient with chromoblastomycosis in Japan and the fungus was described as a new pathogenic species by KANO in 1937.[17]

*Morphology in pathologic tissue.* — The parasite could be observed in the cutaneous lesions of the patient and it was present in both the dermis and epidermis. In the infected epidermal scrapings, as well as in tissue sections, it was described as brownish, round or oval bodies, measuring 2 to 10 microns in diameter, with a dark brown, double-contoured membrane, and a brownish, granular protoplasm. These bodies occurred either singly or in small groups, sometimes in chains of 2 to 5 elements. They were found within giant cells, or free in the tissues, or in the center of microabscesses.

*Gross morphology in culture.* — On 4 per cent maltose agar, growth slow, cultures six weeks old at room temperature measuring 8 mm. in diameter; cultures hemispherical in shape; coal-black in color; surface finely granular and wrinkled; substrate stained by intensely black, diffusible pigment which extends beyond limits of colony.

On 4 per cent grape sugar agar, on mulberry, and on potato plugs, growth essentially similar to that described on maltose agar, cultures most exuberant on potato plugs. On peptone agar, growth poor. In glucose and maltose peptone water, loose floccular colonies developed at bottom of tube, with rest of the liquid clear.

*Microscopic morphology in culture.* — Microscopic characters relatively simple and constant in a large number of laboratory media, both solid and liquid, employed in the study of the fungus. Cultures four days old on "glucose-peptone agar" developed at a temperature of 30° C., showed "short chains of spherical, oval, rod-shaped, clavate, or swollen cells," the chains usually consisting of ten or more elements; branching definite, though rudimentary, dichotomous, sometimes trichotomous, and taking place at the tip or side of the cell, often close to an intercellular septum. Cellular elements of different size, the spherical forms measuring about 10 microns in diameter, the others ranging from 3 to 5 microns in width by 5 to 20 in length; cell membrane thick and dark, becoming double-contoured and more deeply pigmented with age; some cells showing internal septum perpendicular to longitudinal axis.

Large chlamydospores, of "sclerotic" type, present in old cultures;

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[17] We have a culture of *Hormiscium dermatitidis* which was obtained through the *Centraalbureau voor Schimmelcultures* at Baarn, Holland. Comparative studies of this and other similar isolates from chromoblastomycosis are now being conducted in our laboratories.

some of these chlamydospores showing internal septation in one or more planes; some showing constriction at the line of contact of the cell wall with the internal septum; some producing daughter cells by lateral sprouting of the mother element.

On carrot-plug cultures, rudimentary mycelium produced in moderate abundance, each mycelial structure consisting of about ten relatively long and thin articles, measuring 2 to 3 microns in diameter, and 6 to 8 in length.

*Optimum temperature.*—In cultures on maltose agar, optimum growth was produced between 20° and 30° C. At 37° C. the development was very poor, and at 43° C. it was inhibited.

*Sugar fermentation reactions.*—Fermentation activities were tested with maltose, dextrin, levulose, lactose, glucose, arabinose, galactose, saccharose and xylose with negative results in every instance.

*Experimental infections.*—KANO has reported the reproduction of a typical nodule of chromoblastomycosis on the healthy skin of his patient by inoculating, locally, a suspension of *Hormiscium dermatitidis*. The histopathology of the nodule was similar to that of other lesions naturally developed on the patient's skin.

Experimental infections were also produced on three mice, three guinea-pigs, and three rabbits, the inoculum consisting of a suspension of the fungus grown in artificial cultures. The subcutaneous route of inoculation was used in all the animals. However, additional and simultaneous inoculations were made intraperitoneally to the mice and guinea-pigs, and intratesticularly to the rabbits. The subcutaneous inoculations were negative for the mice, but, in the guinea-pigs and rabbits, they were followed by discrete and progressive local indurations. Histopathologically, these indurations were of the type of an infectious granuloma with microabscesses. The intraperitoneal inoculations produced progressive granulomatous nodules in the abdominal organs of all the animals, the lesions taking a tuberculoid character in the mice and guinea-pigs. The testicular lesions developed in the rabbits consisted of a diffuse induration of the organ. Microscopically, the histopathologic reaction was of granulomatous and tuberculoid type, resembling that noted in human lesions, with the infecting parasite constituting a prominent part of the picture.

*Taxonomy.*—This fungus has not shown a sexual phase of reproduction in artificial cultures and, consequently, has been classed among the Fungi Imperfecti. Following the classification of SACCARDO-LINDAU, KANO has included this species in the family *Dematiaceae,* FRIES; subdivision *Phaeosporeae,* SACCARDO; genus *Hormiscium* KUNZE; and, in accordance with OTA's advice, the fungus has been given the provisional name *Hormiscium dermatitidis.*

**Candida-like Species:**— *Source.* — This black, *Candida*-like fungus was repeatedly isolated[18] by BERGER *et al.* (1945) from the cutaneous lesions of a Canadian patient who had been suffering from papillomatous growths over a period of fourteen years. From the original focus of infection, located on the left hand, the disease gradually spread to the opposite hand, the left forearm, the left foot, the right ear, the right buttock, and both cheeks. Histopathologically, the lesions presented the classical picture of chromoblastomycosis, although the infiltrate was sometimes less nodular, and the fungus smaller, more delicate, and distinctly budding.

*Morphology in pathologic tissue.* — In the host tissues, the parasite appeared as yeast-like bodies, the morphology of which was somewhat different in two series of biopsies done at different periods. In the earlier biopsies, these bodies were very abundant, sometimes outnumbering the inflammatory cells, especially in the papillary layer of the dermis. The fungus cells were rather small and delicate, and had a fine membrane, a few of them showing distinct budding, while others would form short, bead-like chains. In biopsies done at a later period, the parasitic cells were not so abundant, and were located in the middle and deeper layers of the dermis. They were coarser, thick-walled, irregular, devoid of budding, generally clustered, often engulfed by giant cells and frequently degenerated.

*Gross morphology in culture.* — In his description of the fungus, as originally isolated, BERGER reports: "Colonies on corn meal and hay infusion agar, Loeffler's serum and blood agar, remain small (3 to 4 mm.), and die soon on the two latter media, but are larger on glycerolated potato and carrot, and on Sabouraud's agar (4 to 7 mm.), and still larger on Lowenstein's and Petragnani's media (up to 15 mm.). On Sabouraud's agar, they are dark brown from the beginning, and half-spherical; on the other media, they are coal-black; they are always moist, of creamy consistence and glossy. . . ." "In potato water (LANGERON and TALICE), small, fluffy colonies rest on the bottom, and the liquid contains a great number of single or budding yeast cells; there is no veil."

"Cultures made from a biopsy in 1944 yielded brown to black colonies which grew much more slowly, and were rough and dry, and presented microscopical features differing from those of colonies obtained before."

*Microscopic morphology in culture.* — Differences in microscopic morphology were likewise noted between the early and late isolations of the fungus. BERGER states that cultures of the early isolations "at *p*H 7 are predominantly formed by yeast-like cells and of only a scant pseudomycelium; at lower *p*H's, pseudomycelial branching filaments extend on the surface and into the underlying medium, and bear clusters of pseudoconidial blastospores; there are no aerial hyphae." In cultures obtained from a later biopsy (1944), the "fundamental characters are still yeast-like, but the cells are larger and thicker, often double-walled; outlines often irregular, and many elements show internal septations in different planes. The resulting multicellular structures become very complicated, and are made up of intricately intertwined, irregularly curved, and anastomosing, rather thick threads of clustered elements which grow in all directions." These ". . . cells may contain one or more round lipoid inclusions. . . ." They "may be considered" as "sclerotic cells and are obviously a variant of the initially yeast-like pseudomycelial fungus."

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[18] A culture of this species was kindly sent to us by Dr. L. BERGER. Comparative studies of this and other similar organisms isolated from cases of chromoblastomycosis are now being conducted in our laboratories.

"In hanging drop and slide cultures, many budding cells may be seen which remain attached to the mother cell, and form, by successive buddings, moniliform strands of various lengths; the latter become pseudomycelial through elongation of the individual elements. Apical blastospores appear and give in turn rise to pseudomycelial threads, but the branches are few and the resulting arborization is, therefore, rather simple. Some blastospores show intensive budding of many small conidia-like blastospores, which form soon more or less large clusters of easily detached cells. There is no trace whatsoever of conidiophores or of any other specialized apparatus of sporulation, although many colonies were kept alive for over two years."

*Effects of temperature, anaerobiosis, and pH on growth.* — BERGER's organism grows equally well at room and incubator temperatures; it does not grow in the depth of agar stabs; at *p*H 7 its morphology resembles that of the yeast, and at lower *p*H's, the cultures become filamentous.

*Biochemical reactions.* — The fungus does not liquefy gelatin and has no action on milk. It acidifies agar media containing glucose, fructose, mannose, galactose, cellobiose, and xylose respectively, but there is no gas formation in the corresponding liquid sugar media.

*Literature Cited:* —

DE ALMEIDA, E. Hospital, Río de Janeiro *21:* 467, 1942.

DE ALMEIDA, F. P. Rev. biol. e hyg. *5:* 95, 1934.

DE ALMEIDA, F. P. Mycologia Medica, Comp. Melhoramentos de São Paulo, 1939.

ARÊA-LEÃO, A. E., A. CURY and M. THIAGO DE MELLO. Brasil-Med. *59:* 393, 1945.

AZULAY, R. D. Cromoblastomicose. "Clínica e Laboratorio," Thesis. 69 pp. Río de Janeiro, 1944.

AZULAY, R. D. J. Invest. Dermat. *6:* 281, 1945.

BALIÑA, P., P. BOSQ, P. NEGRONI and M. QUIROGA. Rev. argent. dermatosif. *16:* 369, 1932.

BARROS-BARRETO, A. L. DE. Hospital, Río de Janeiro *23:* 577; 743, 1943.

BERGER, L., M. BEAUDRY and E. GAUMOND. Canad. M. A. J. *53:* 138, 1945.

BINFORD, C. H., G. HESS and C. W. EMMONS. Arch. Dermatol. & Syphilol. *49:* 398, 1944.

BOGGINO, L. Novena reunión soc. argent. de pat. reg. *2:* 1329, 1937.
BONNE, C. 1928, cited by BARROS-BARRETO, 1943, *o. c.*
BRICEÑO-IRAGORRI, L. Rev. Clin. Luis Razzeti *1:* 108, 1938.
BRICEÑO-IRAGORRI, L. 1939, cited by AZULAY 1944, *o. c.*
BRUMPT, E. Précis de Parasitologie, 3ᵉ édition, Masson et Cie, Paris, p. 1105, 1922.
CARINI, A. Ann. Inst. Pasteur *24:* 157, 1910.
CARRIÓN, A. L. Puerto Rico J. Pub. Health & Trop. Med. *10:* 543, 1935.
CARRIÓN, A. L. Puerto Rico J. Pub. Health & Trop. Med. *11:* 663, 1936.
CARRIÓN, A. L. Puerto Rico J. Pub. Health & Trop. Med. *14:* 37, 1938.
CARRIÓN, A. L. Puerto Rico J. Pub. Health & Trop. Med. *15:* 340, 1940.
CARRIÓN, A. L. Mycologia *34:* 424, 1942.
CARRIÓN, A. L. and C. W. EMMONS. Puerto Rico J. Pub. Health & Trop. Med. *11:* 114, 1935.
CARRIÓN, A. L. and E. KOPPISCH. Puerto Rico J. Pub. Health & Trop. Med. *9:* 169, 1933.
CARRIÓN, A. L. and M. F. PIMENTEL-IMBERT. Puerto Rico J. Pub. Health & Trop. Med. *13:* 522, 1938.
CASTRO-PALOMINO, J. and J. ALFONSO-ARMENTEROS. Rev. de med. y cir., Habana *46:* 217, 1941.
CONANT, N. F. Mycologia *29:* 597, 1937.
CONANT, N. F. and D. S. MARTIN. Am. J. Trop. Med. *17:* 553, 1937.
DODGE, B. O. Mycologia *28:* 284, 1936.
DODGE, C. W. Medical Mycology, C. V. Mosby Co., St. Louis, 1935.
DOX, A. W. 1910, cited by THOM and CHURCH 1926, *o. c.*
EMMONS, C. W. and A. L. CARRIÓN. Puerto Rico J. Pub. Health & Trop. Med. *11:* 703, 1936.
EMMONS, C. W. and A. L. CARRIÓN. Mycologia *29:* 327, 1937.
FLEMING, R. S. and F. QUEEN. Am. J. Clin. Path. *16:* 66, 1946.
FONSECA, O. DA and A. E. DE ARÊA-LEÃO. Compt. rend. Soc. de biol. *89:* 762, 1923.
FONSECA, O. DA and A. E. DE ARÊA-LEÃO. Rev. med.-cir. do Brasil. *38:* 197, 1930.
GOMES, J. M. Bol. Soc. de med. e cir. de São Paulo *4*· 26, 1921.
GOMES, J. M. and S. B. PESSÔA. Brasil-med. *43:* 255, 1929.
KANO, K. Arch. f. Dermat. u. Syph. *176:* 282, 1937.
KEENEY, E. L., L. AJELLO and E. LANKFORD. Bull. Johns Hopkins Hosp. *75:* 377, 1944.
KEENEY, E. L., L. AJELLO and E. LANKFORD. Bull. Johns Hopkins Hosp. *75:* 393, 1944.
KEENEY, E. L., L. AJELLO and E. LANKFORD. Bull. Johns Hopkins Hosp. *75:* 410, 1944.
KRESS *et al.* 1925, cited by MARTIN 1938, *o. c.*
LAGERBERG *et al.* 1927, cited by VAN BEYMA 1943, *o. c.*
LANE, C. G. J. Cutaneous Dis. *33:* 840, 1915.
LANGERON, M. Ann. de parasitol. *7:* 145, 1929.
LEVY, B. and B. BLACK-SCHAFFER. Am. J. Trop. Med. *25:* 117, 1945.
MACKINNON, J. E. Arch. urug. de med., cir. y especialid. *5:* 201, 1934.
MACKINNON, J. E. Ann. de parasitol. *14:* 78, 1936.
MARTIN, D. S. Am. J. Trop. Med. *18:* 421, 1938.
MARTIN, D. S., R. D. BAKER and N. F. CONANT. Am. J. Trop. Med. *16:* 593, 1936.
MAZZA, S. and F. L. NIÑO. Novena reunión Soc. argent. de pat. reg. *3:* 1571, 1939.
MAZZA, S. and I. WOFCY. Novena reunión Soc. argent. de pat. reg. *3:* 1669, 1939.

MEDLAR, E. M. J. Med. Research *32:* 507, 1915.

MELIN, E. and J. A. NANNFELDT. 1934, cited by MARTIN, 1938 *o. c.*

MERIIN, J. Arch. f. Dermat. u. Syph. *162:* 300, 1930.

MERIIN, J. Arch. f. Dermat. u. Syph. *166:* 722, 1932.

MERIIN, J. Ann. de dermat. et syph. *9:* 122, 1938.

MONTPELLIER, J. and A. CATANEI. Ann. de dermat. et syph. *8:* 626, 1927.

MONTPELLIER, J. and A. CATANEI. Arch. Inst. Pasteur d'Algérie *22:* 13, 1944.

MOORE, M. J. Invest. Dermat. *5:* 411, 1942.

MOORE, M. and F. P. DE ALMEIDA. Rev. biol. e hyg. *6:* 94, 1935.

MOORE, M. and F. P. DE ALMEIDA. Ann. Missouri Bot. GARD. *23:* 543, 1936.

MOORE, M. and F. P. DE ALMEIDA. Rev. biol. e hyg. *7:* 59, 1936.

MOORE, M. and P. MAPOTHER. Arch. Dermatol. & Syphilol. *41:* 42, 1940.

MOORE, M., Z. K. COOPER and R. S. WEISS. J. A. M. A. *122:* 1237, 1943.

NAUCK, E. G. Rev. méd. german.-ibero-am. *4:* 671, 1931.

NEGRONI, P. Rev. d. Inst. bact. *7:* 419, 1936.

NEGRONI, P. Morfología y Biologia de los Hongos, "El Ateneo," Buenos Aires, p. 234, 1938.

O'DALY, J. A. Rev. san. y asist. social *8:* 655, 1943.

OTA, M. Jap. J. Dermat. & Urol. *28:* 16, 1928.

PEDROSO, A. and J. M. GOMES. Ann. paulist, de med. e cir. *11:* 53, 1920.

PEREIRA, O. B. "Mycologia das cromomycoses." Thesis. 184 pp. Porto Alegre, Brasil, 1938.

POZOJEWA, N. G. Dermat. Wchnschr. *90:* 615, 1930.

REDAELLI, P. Gior. ital. di dermat. e sif. *76:* 253, 1935.

RUDOLPH, M. Arch. f. Schiffs. u. Tropen-Hyg. *18:* 498, 1914.

SABOURAUD, R. Les Teignes. Masson et Cie., Paris, p. 114, 1910.

SATINA, S. 1917, cited by B. O. DODGE 1936, *o. c.*

SIMSON, F. W. Mycologia. *38:* 432, 1946.

SIMSON, F. W., C. HARINGTON and J. BARNETSON. J. Path. & Bact. *55:* 191, 1943.

SNOW, J. S., E. S. WEDDING and W. J. TOMLINSON. Arch. Dermatol. & Syphilol. *51:* 90, 1945.

TAKAHASHI, Y. Jap. J. Dermat. & Urol. *41:* 31, 1937.

TAKAHASHI, Y. Jap. J. Dermat. & Urol. *41:* 53, 1937.

TERRA, F., M. TORRES, O. DA FONSECA and A. E. DE ARÊA-LEÃO. Brasil-med. *36:* 363, 1922.

THOM, C. J. Washington Acad. Sci. *30:* 49, 1940.

THOM, C. and M. CHURCH. The Aspergilli. Williams & Wilkins Co., Baltimore, p. 39, 1926.

TIBIRIÇÁ, P. Q. T. Anatomía Patológica Dermatite Verrucosa Cromomicótica. Thesis. 85 pp. Emprêsa Gráfica da "Revista dos Tribunais." São Paulo, 1939.

TSCHERNJAWSKI, J. Arch. f. Dermat. u. Syph. *157:* 196, 1929.

VAN BEYMA THOE KINGMA, F. H. Antonie van Leeuwenhoek, J. Microbiol. Serol. *9:* 51, 1943.

VUILLEMIN, P. 1931, cited by BARROS-BARRETO 1943, *o. c.*

WEIDMAN, F. D. and L. H. ROSENTHAL. Arch. Dermatol. & Syphilol. *43:* 62, 1941.

WILSON, S. J., S. HULSEY and F. D. WEIDMAN. Arch. Dermatol. & Syphilol. *27:* 107, 1933.

WOLLENWEBER, H. W. and O. APPEL. 1913, cited by VAN BEYMA 1943, *o. c.*

## *Chapter 4*

# BIOLOGY OF PITYROSPORUM OVALE

*by*

RHODA W. BENHAM

**Introduction:**—*Pityrosporum ovale* is a yeast-like organism, oval or elongate in form, which is found consistently in dandruff scales. It may easily be demonstrated in methylene blue preparations of the scales. In such preparations the organism appears as small blue staining bodies, some dense, some vacuolated, of the characteristic round or elongated bottle shape the average size being $0.8 \times 2\mu$ ranging from $0.8\text{-}1.5 \times 2\text{-}3\mu$. To RIVOLTA (1873) goes the credit for first describing this fungus, in his Parasiti Vegetali. He described it, under the name *Cryptococcus psoriasis,* as a rounded cell with a double contoured membrane which does not produce a nucleus. He had found it in a case of psoriasis vulgaris. The next year MALASSEZ (1874) gave a more precise description of the organism which he found in scales from alopecia areata; he considered the organism to be pathogenic. His so-called "spores" budded and were ovoid, rarely spherical, showing at all times some differences as to form and size. He found three types of cells, (*1*) which budded and was large, 4-5$\mu$ in diameter, (*2*) which also budded but was smaller, about 2$\mu$ in diameter, (*3*) did not bud and the diameter was less than 2$\mu$. The last mentioned he regarded as daughter cells. MALASSEZ found similar "spores" in simple pityriasis, but these were always spherical and more numerous, these he also considered pathogenic. KLAMAN (1884) without referring to the work of MALASSEZ came to the same conclusion concerning these "spores" and their relation to pityriasis. LOGIE found the "spores" in the epidermal scales and likewise concluded that they were pathogenic. A number of the early students of these conditions held to the view that this organism was the cause of the lesion in which it was found. Among these may be listed COURRÈGES, HARDY (1876) and HODARA (1894). Others, namely HORAND (1874, '75) and NYSTROM (1875), held the view that it was a harmless saprophyte.

BIZZOZERO (1884) found small spherical cells, 2.5-5$\mu$ in diameter, in scales from regions of the body covered with hair: the scalp, chin, lip, and pubis. To these he gave the name *Saccharomyces sphaericus.* He also found smaller ovoid cells $2\text{-}2.5 \times 3.5\mu$ which he considered identical with the "spores" of MALASSEZ and which he called *Saccharomyces ovalis.* Since these cells were found to occur so abundantly on so many people, he did not consider them to possess any pathogenic significance. BOECK (1886) noted these "spores" both in pityriasis capitis and eczema marginatum and considered them to be merely saprophytes. UNNA (1891), observing what apparently were the same structures in all cases of pityriasis capitis, thought they were a bacillus and introduced the name "flaschen bacillus" or bottle bacillus, a name which clings to this day and is made familiar in many advertisements offering a cure for "infectious dandruff" so called. HODARA (1894) agreed with UNNA, and in addition, claimed to have cultured the bacillus, but reported that it would not live long on agar. SABOURAUD (1902, '04) reported he always found the "spore" of MALASSEZ in pityriasis and in the scales of exfoliated epidermis; he considered them to be the etiological agent in these conditions. JACQUET and RONDEAU (1905) and DARIER (1907) were opposed to the microbic etiology of pityriasis as was TIÈCHE (1908) who claimed that seborrhea and excessive desquamation are seen in all cutaneous infections. This controversy on the etiological significance of *Pityrosporum* in seborrheic dermatitis and pityriasis capitis emphasized the need for obtaining cultures of these tiny yeast-like organisms; many attempts were made to do so. The task proved to be more difficult than would be anticipated from the frequency with which the organism is found on the scalp.

**Attempts to Grow P. ovale in Culture:**— HODARA (1894) stated that UNNA (1891) was the first to culture *Pityrosporum,* that he was able to obtain subcultures on agar, but that they did not live long as the medium was not favorable. HODARA says that he himself obtained primary cultures but was never successful in subculturing.

VAN HOORN (1895) claimed that PEKELHARING, a Dutch worker, preceded UNNA in culturing from hairs in cases of simple pityriasis the "spore" of MALASSEZ and of BIZZOZERO. OUDEMANS, a botanist, studied this fungus and classed it in the genus *Saccharomyces,* naming it *Saccharomyces capillitii.* He also designated under the names *S. sphaericus* and *S. ovali* yeasts which he considered identical to each other and to those of PEKELHARING. The descriptions he gave are vague and much doubt exists as to whether the organisms were actually species of *Pityrosporum.* SABOURAUD (1904) held this opinion and announced that the "spore" of MALASSEZ had not been cultured on artificial medium stating that he himself had tried it on many media without success. He claimed that the organism belongs to the lower cryptograms and, because of its habit of budding, with the blastomycetes.

Later reports of the successful cultivation of this fungus included those of MARZINOWSKI and BOGROW (1907) and DOLD (1910). The description by these authors, however, leaves one in doubt as to the identity of their cultures. MEIROWSKY (1911) stated that the bottle bacillus of UNNA is not a bacillus, but a yeast and that it may easily be cultured on a medium containing lanolin, but gave no details concerning the cultures. KRAUS (1913) also obtained a culture on a medium containing lanolin. His description of the organism stated that, as seen in culture, it appears identical with that in the scales. He did, however, mention a mycelium and this at once makes one doubtful as to the identity of his culture.

To come to the more recent studies, CASTELLANI (1925) isolated from a case of stomatitis an organism which he named *Cryptococcus graciloides*. OTA and HUANG (1933) felt that CASTELLANI in actuality had isolated *Pityrosporum*. TEMPLETON (1926) seems also to have cultured *Pityrosporum* from scales of pityriasis simplex, on media containing oleic acid. ACTON and PANJA (1927) obtained what seems to be a valid culture on a medium containing glycerine. The culture of MACLEOD and DOWLING (1928) was obviously not *Pityrosporum*, this was so stated by DOWLING in a later publication (1939). The yeast isolated by BENEDEK (1930) was very different from the *Pityrosporum*, although he thought it identical, and tried to explain the discrepancies by saying the organism was different in culture from that found in the scales. MOORE's (1935) strain has been shown to be a *Cryptococcus* (*Torulopsis*) belonging to BENHAM's group III. It grows very readily and abundantly on the usual media, quite the opposite of *Pityrosporum*.

**Necessity of Fatty Substances in Culture Media:**— It would seem that it was not until the important paper by OTA and HUANG (1933) that the question of the culture of the organism, seen so abundantly in dandruff scales, was cleared up. The authors studied the culture of ACTON and PANJA (1927) and the *Cryptococcus graciloides* of CASTELLANI and found that these were identical with their own cultures. From their descriptions, and the difficulty with which the cultures were obtained, there is no doubt as to the identity of the cultures with *Pityrosporum ovale*. They found it necessary to add butter or lecithin to the medium. On untreated media, they obtained cultures of ordinary yellow or red yeasts. TEMPLETON (1926) probably had the true organism but failed to carry on subcultures.

BENHAM (1939) was able to isolate eight strains of *P. ovale* and to maintain five of them in subculture on a medium to which was added fats and fatty substances. These cultures appeared identical with the organism seen in the scales and with the description of the cultures given by OTA and HUANG. The fats were added to the medium in the form of ether extracts or solutions pipetted over the surface of the agar (wort) in tubes. The ether evaporated leaving a thin film of the fat on the surface of the agar. Ether extracts of crude lanolin and butter were found to be most effective. EMMONS (1940) corroborated these findings by successfully culturing and maintaining in subculture a number of strains which corresponded in every way to the description of *P. ovale* and which appeared identical with Benham's strains. EMMONS isolated his strains by planting the scales in acid dextrose broth containing 23 to 44% glycerine, and incubating at 30 to 37° C. His subcultures were maintained on media treated with ether extracts of lanolin or oleic acid. It will be noticed that all who were successful in culturing *P. ovale* did so only when some fatty substance was added to the medium. BENHAM (1941), using a synthetic medium, proved that this organism will not grow unless fats are added, thus indicating for the first time a case in which a micro-organism requires a fatty substance for continued growth.

**Classification and Nomenclature:**— The fact that *P. ovale* was first described by RIVOLTA (1873) under the name *Cryptococcus psoriasis* indicated that the organism was yeast-like. However, MALASSEZ (1874) described it merely as a spore, the "spore" of MALASSEZ. UNNA (1891) added further complications by terming it a bacillus and the organism is still commonly called the bottle bacillus. It is to SABOURAUD that we owe the name *Pityrosporum,* from the Greek *Pityron* meaning scale. He gave it the specific name *Malassezi* in honor of MALASSEZ. BRUMPT (1936) accepts the name *Pityrosporum,* but in a new combination, using as the specific name *ovale,* originating from BIZZOZERO (1884). This is the commonly accepted name for the organism.

Since the organism is yeast-like, reproducing by budding, and does not produce a mycelium or ascospores, it would of necessity be placed in the *Torulopsidoideae* near the genus *Cryptococcus* (*Torulopsis*). Since it differs from this in size and manner of vegetative reproduction it seems better to keep it as a distinct entity under the now familiar name *Pityrosporum.* It reproduces by a process between budding and septation much as found with the genus *Saccharomycodes* and therefore may be an imperfect form of this genus.

**Cultural Characteristics:**— Wort agar has been found to be a suitable medium for the isolation of *Pityrosporum ovale* from dandruff scales. If fats are added as described above, this same medium will serve for subcultures. Media which have begun to dry out are not satisfactory for supporting growth of this micro-organism. In the original cultures growth is first noted about the scales, cream to tan or orange colored and dry in appearance. If a suspension of this growth is pipetted over the surface of wort agar to which oleic acid has been added growth will appear in four or five days. It appears first as tiny cream-colored colonies which later fuse, becoming dry and wrinkled with the color varying from cream to tan to orange. In a synthetic liquid medium (described later in this paper) in flasks, *Pityrosporum* grows both in granules which settle to the bottom of the flask and as a film or scum covering the surface and spreading up the sides of the flask above the level of the medium. The earliest growth appears as fine particles in or attached to globules of oleic acid which float on the surface.

**Microscopic Characteristics:**—*Pityrosporum ovale* characteristically has several forms in culture though usually it appears as an elongate structure of 2-3 × 4-5$\mu$ (*see* Fig. 1). It frequently appears two-celled, one of the cells being smaller suggesting a bud (Fig. 1A). In some instances the bud has elongated forming a neck-like projection (Fig. 1B). This is the so-called "bottle form." Often the cells appear joined by a septum, indicating that the reproduction is intermediary between budding and septation, as described for the genus *Saccharomycodes*. These yeasts form buds which become separated from the parent cell by the formation of a septum at the base rather than by constriction of the base. This is a rare group and only one species is well known, *Saccharomycodes Ludwigii*. This species forms ascospores and is much larger than *Pityrosporum ovale*.

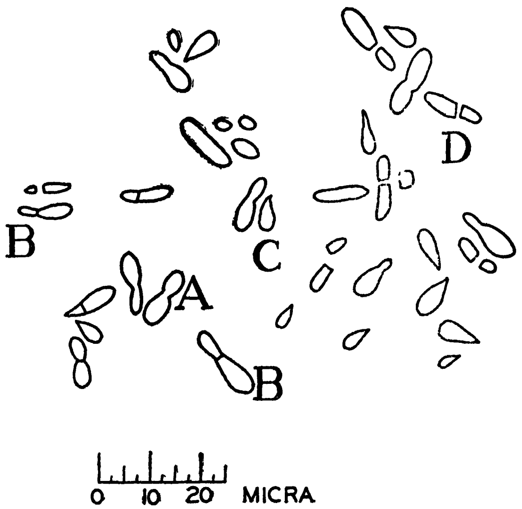


FIGURE 1.—*Pityrosporum ovale*, organism grown on a wort + oleic acid medium. A, bud; B, "bottle" form; C, parallel pairs; D, separating cells.

Sometimes the cells lie in parallel pairs (Fig. 1C). The daughter cell, after separating from the mother cell, continues to lie near it (Fig. 1D). There is no indication of mycelial development and no multiple budding. Large aggregate masses form by the clumping of cells. Individual cells may contain small vacuoles. Some spherical thick walled cells are seen. These may be in the nature of resistant cells and are characteristic of most yeasts.

**Fats and Fatty Acids in the Nutrition of P. ovale:**—*Pityrosporum ovale* has been shown to require a fatty substance for continued growth. Of a number of fats tried, crude lanolin and butter seem to be the most effective. Of the pure fatty acids tested, oleic gave the best results. Oleic acid was used by FLEMING (1909) for cultivation of the acne bacillus and has since been used in a number of bacteriological mediums. COHEN and MUELLER (1940) have shown its importance for *C. diphtheriae.* However, *Pityrosporum* is probably the first micro-organism reported to need fats or fatty acids as an indispensable factor for growth.

**Culture of P. ovale in a Synthetic Medium:**— If oleic acid is supplied, *Pityrosporum ovale* will sometimes grow feebly on an inorganic solution similar to Currie's medium containing $NH_4Cl$, $KH_2PO_4$, $MgSO_4$, and dextrose (BENHAM, 1945). Substitution of asparagin as a nitrogen source caused increased growth. If thiamin is added, some growth will be obtained regularly even with $NH_4Cl$ as the nitrogen source, and with asparagin there will be an increased yield. Dextrose regularly increases growth when asparagin is used as the nitrogen source, and with thiamin gives yields practically equal to the best obtained. When dextrose is added to the series with $NH_4Cl$ as a nitrogen source, with or without thiamin, there is no increase compared with parallel flasks without dextrose.

The addition of ethyl oxaloacetate causes a striking acceleration of growth (*see* Table 1). With oxaloacetate, and asparagin as a nitrogen source, the yields, determined as dry weight of the fungus, are equal to or slighly higher than those with asparagin plus thiamin. With $NH_4Cl$, the yields are about as good as those with asparagin. The addition of both thiamin and oxaloacetate give no greater average yield than oxaloacetate alone. The addition of dextrose increases

TABLE 1.—*Effect of Thiamin, Oxaloacetic Acid, and Nitrogen Source on the Growth of P. ovale:*—

| | *With Dextrose* | | *Without Dextrose* | |
|---|---|---|---|---|
| MEDIUM* | NO. OF EXPTS. | AVERAGE YIELD IN MG. DRY WT. | NO. OF EXPTS. | AVERAGE YIELD IN MG. DRY WT. |
| Asparagin | 15 | 44 | 8 | 24 |
| Asparagin + Thiamin ($B_1$) | 15 | 123 | 7 | 57 |
| Asparagin + Oxaloacetic | 9 | 174 | 7 | 96 |
| Asparagin + Oxaloacetic + $B_1$ | 4 | 114 | 3 | 72 |
| Ammonium Chloride | 12 | 6 | 8 | 4 |
| $NH_4Cl + B_1$ | 8 | 20 | 4 | 24 |
| $NH_4Cl$ + Oxaloacetic | 7 | 145 | 4 | 108 |
| $NH_4Cl$ + Oxaloacetic + $B_1$ | 3 | 171 | 3 | 104 |

* The modified Currie's Solution with additions as listed.

the yield both with asparagin and $NH_4Cl$ when oxaloacetate is present.

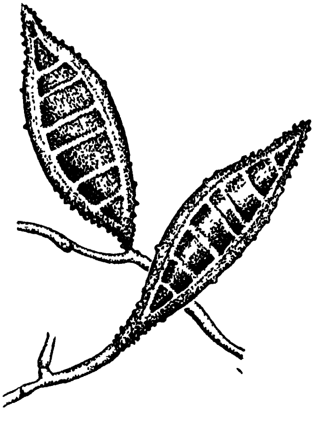
To prevent the carrying over of any thiamin with the inoculum, the suspensions used for seeding must be dilute and the cells carefully washed. Only two to three drops of such a suspension should be used to each flask containing 50 cc. of medium.

These facts point to *Pityrosporum ovale* as an interesting organism from the standpoint of accessory food substances. As it has definite requirements of its own, it may help in an understanding of the function of some of these substances. For example, it would seem that $NH_4Cl$ and ethyl oxaloacetate can be substituted for asparagin and thiamin in the cultivation of *Pityrosporum ovale.* It appears that if a suitable keto acid is supplied, neither thiamin nor an amino acid is necessary. On the other hand, the asparagin may furnish both the carbon and nitrogen source needed by the organism, yielding ammonia and a carbon skeleton, probably oxaloacetate. Thiamin increases growth when added to the asparagin by hastening the production of the carbon skeleton in some way. When this carbon skeleton is supplied as oxaloacetate, thiamin is no longer necessary. It will be interesting to continue the study of the nutrition of *P. ovale,* especially using fatty acids other than oleic, and to investigate the role played by the various vitamins.

## *Literature Cited:* —

ACTON, H. W. and J. PANJA. Indian Med. Gaz. *62:* 603, 1927.
BENEDEK, T. Zentrlb. f. Bakt., I. *116:* 317, 1930.
BENHAM, R. W. J. Invest. Dermat. *2:* 187, 1939.
BENHAM, R. W. Proc. Soc. Exp. Biol. & Med. *46:* 176, 1941.
BENHAM, R. W. Proc. Soc. Exp. Biol. & Med. *58:* 199, 1945.
BIZZOZERO, J. Virchows Arch. f. path. Anat. *98:* 411, 1884.
BOECK, C. Analyse dans Monatschr. f. prakt. Dermat. *5:* 90, 1886.
BRUMPT, E. Précis de Parasitologie *2:* 1788. Paris, Masson et Cie, 1936.
CASTELLANI, A. J. Trop. Med. *28:* 217, 1925.
COHEN, S. and J. H. MUELLER. Proc. Soc. Exp. Biol. & Med. *45:* 244, 1940.
COURRÈGES. Quoted by OTA and HUANG.
DARIER, J. Ann. de dermat. et syph. *8:* 3, 1907.
DOLD, H. Parasitology. *3:* 279, 1910.
DOWLING, G. B. Brit. J. Dermatol. *51:* 1, 1939.
EMMONS, C. W. Public Health Repts. *55:* 1306, 1940.
FLEMING, A. Lancet. *1:* 1035, 1909.
HARDY. Ann. de dermat. et syph. *8:* 401, 1876-1877.
HODARA, M. Monatschr. f. prakt. Dermat. *18:* 573, 1894.
HORAND, M. A. Ann. de dermat. et syph. *6:* 408, 1874-1875.
HORAND, M. A. Ann de dermat. et syph. *7:* 5, 1875-1876.
JACQUET, L. and RONDEAU. Ann. de dermat. et syph. *6:* 33, 1905.
KLAMAN. Allg. Med. Central-Zeitung, No. 23, 1884.
KRAUS, A. Arch. f. Dermat. u. Syph. *116:* 723, 1913.
LOGIE. Quoted by OTA and HUANG.
MACLEOD, J. M. H. and G. B. DOWLING. Brit. J. Dermatol. *40:* 139, 1928.
MALASSEZ, R. Arch. de physiol. Serie 2, *1:* 451, 1874.
MARZINOWSKI, E. J. and S. L. BOGROW. Arch. f. Dermat. u. Syph. *86:* 215, 1907.
MEIROWSKY, E. Arch. f. Dermat. u. Syph. *108:* 129, 1911.
MOORE, M. Arch. Dermatol. & Syphilol. *31:* 661, 1935.
NYSTROM, A. Ann. de dermat. et syph. *7:* 440, 1875-1876.

OTA, M. and P. T. HUANG. Ann. de parasitol. *11:* 49, 1933.
RIVOLTA, S. Parasiti Vegetali. Torino, 1873.
SABOURAUD, R. Séborrhée, Acnés, Calvitie. Paris, Masson, 1902.
SABOURAUD, R. Pityriasis et Alopécie Pelliculaires. Paris, Masson, 1904.
TEMPLETON, H. J. Arch. Dermatol. & Syphilol. *14:* 270, 1926.
TIÈCHE. Arch. f. Dermat. u. Syph. *92:* 125, 1908.
UNNA, P. G. Monatschr. f. prakt. Dermat. *13:* 225, 1891.
UNNA, P. G. Monatschr. f. prakt. Dermat. *18:* 257, 1894.
VAN HOORN, W. Monatschr. f. prakt. Dermat. *20:* 545, 1895.



## *Chapter 5*

# BIOLOGY OF COCCIDIOIDES

*by*

C. W. EMMONS

**Introduction:** — *Coccidioides immitis* is perhaps the best known of the fungi which cause generalized mycoses of man, yet it challenges the student with many unanswered questions. For several years after its discovery it was classified among the Protozoa. OPHULS and MOFFITT (1900) convincingly demonstrated its fungus nature, yet its anomalous life cycle has led modern investigators to assign it to positions as far apart in taxonomic mycology as the Ascomycetes and Phycomycetes. Its relationships in the latter group (where it undoubtedly belongs) are still undetermined. The disease which it causes is not transmitted from man to man, and it seems certain from epidemiological and case history studies that most infections follow inhalation of spore-laden dust, yet it is not proved whether the fungus normally grows as a saprophyte in soil or whether it is primarily a disease of rodents by which the soil is contaminated. The disease is geographically limited to relatively small endemic areas in which it may be very common, yet we do not know the ecological factor which imposes this limitation unless it be the required presence of certain species of rodents which, unlike more widely distributed species, are properly balanced as to susceptibility and resistance as to provide a suitable reservoir. Besides its generalized and highly fatal form the disease occurs as a benign, self-limited, respiratory infection, as shown by DICKSON (1937, 1938), GIFFORD (1936), SMITH (1940, 1943), and others; yet the very mild, subclinical form of infection by which persons become sensitized and apparently immunized passes unrecognized in most infant residents of an endemic area.

**History:** — The mycosis caused by *C. immitis* was first reported from Argentina by POSADAS and WERNICKE in 1892. They described the disease as protozoan. The second and third recorded cases were studied in southern California by RIXFORD and GILCHRIST (1896) who, seeing a resemblance to *Coccidium,* described the disease as coccidioidal pseudotuberculosis, and named the organism *Coccidioides immitis.* OPHULS and MOFFITT (1900) reported the third North American case and, having isolated the organism in culture, described its various growth phases and proved that it was a fungus. OPHULS (1905) supplemented the description of the parasitic growth phase of the fungus given by RIXFORD and GILCHRIST by a description, complete in essential details, of the growth of the fungus in tissue, its production of spores by the formation of cleavage furrows, the transformation of this growth phase to that of a mold when the fungus is planted on agar, and the production of chlamydospores in culture. Many subsequent investigators have confirmed OPHULS' observations and corrected and supplemented them in details.

Most of the early observations of the mycosis were made in southern California and especially in the San Joaquin Valley. Many of the reported cases from South America were incorrectly diagnosed and were, in fact, paracoccidioidal granuloma or South American blastomycosis, as later South American investigators pointed out. Only a few authentic South American cases are recorded. As the disease became better known and was searched for it was found in southern Arizona and western Texas. The extensive use of coccidioidin in skin-testing by C. E. SMITH has to a considerable extent determined the limits of the disease in this general region. The observations of ARONSON, SAYLOR and PARR (1942) have indicated that there is a high incidence of the mycosis in southern Arizona. The few reported cases from other parts of the United States are in some cases in persons who in all probability were infected while traveling or residing within the known endemic areas of the disease or who may have been infected from packing materials, food products or similar material originating in those areas. A possible endemic focus may exist in Italy. It is generally recognized, however, that the important endemic area is the arid region of southwestern United States.

**Primary Coccidioidomycosis:** — Until 1937 the mycosis, generally known as coccidioidal granuloma, was recognized as a generalized infection having a mortality rate of 40 to 50%. At that time GIFFORD (1936) and DICKSON (1937), investigating a relatively mild respiratory disease common in the San Joaquin Valley and known to the layman as Valley fever, desert rheumatism, etc., proved that this was the primary form and by far the commonest manifestation of infection by *C. immitis*. DICKSON proposed that this be called *primary coccidioidomycosis*. Infection follows inhalation of wind-blown spores and occurs following dust storms and in people exposed in such dusty occupations as fence or road building and picking dusty grapes or cotton. Many infections, as revealed by coccidioidin skin tests are subclinical. This appears to be usual in the case of infants or young children who are native to the endemic area. ARONSON, SAYLOR and PARR (1942) showed that in southern Arizona as many as 97% of Indian children may react to coccidioidin, yet the disease remains unrecognized in this age group and population. On the other hand, in adults entering the endemic area the primary infection is often severe and may be characterized by cough (usually nonproductive), pleural pain, malaise and loss of appetite, fever, chills, nightsweats, headache, and backache. There may be a transient rash. Some or all of these symptoms may appear 10 to 14 days after exposure. From 2 to 20 days after the acute respiratory stage the lesions of erythema nodosum and sometimes of erythema multiforme may appear. This constitutes the classical picture of "Valley fever" (primary coccidioidomycosis).

Roentgenograms may show a widening of the hilar shadows, pneumonic infiltration, nodular lesions in the lung parenchyma, small pleural effusions, and occasionally thin-walled cavities, which close spontaneously. Despite the dramatic onset and severity of the symptoms in some cases, the prognosis in primary coccidioidomycosis is very good. Cavities, when present, heal much more quickly than in tuberculosis and the pulmonary lesions may completely disappear, or calcification may follow healing. In most cases there are no sequelae and the only evidence of a past infection is the persistence of skin sensitivity as revealed by the coccidioidin intradermal test.

**Progressive Coccidioidomycosis:** — In perhaps 0.2% of persons infected with *C. immitis*, *secondary* or *progressive coccidioidomycosis* develops, usually within a few weeks, but occasionally after a lapse of several years. This is the generalized form of the disease which was first recognized (coccidioidal granuloma). It presents many clinical variations, involving as it does almost any organ with the usual exception of the gastro-intestinal tract. The infection extends from the lungs to involve the other visceral organs, brain, meninges, bones, joints, subcutaneous tissues, and skin. Fistulas draining deep-seated lesions develop; there are often enormous sacculate subcutaneous abscesses, and ulcers of the skin are frequent. In rare cases primary skin lesions develop. In the exudate from lesions the fungus can be found, often in great numbers. The mortality rate is high but spontaneous arrest of the infection occurs in some cases as shown by Cox and SMITH (1939).

**Laboratory Diagnosis:** — Diagnosis is made by the laboratory demonstration of the fungus and its isolation in culture. Pus or sputum can be examined by mounting a small drop (with or without an equal amount of 10% NaOH) on a slide under a cover slip and searching it for the presence of the large spherical cells of the fungus which reach a diameter of 60$\mu$ (larger in exceptional cases). There may be some doubt about the identity of the organism unless mature cells containing endospores can be found. The fungus grows rapidly on a wide variety of media and isolation in culture is therefore relatively easy if the material is not heavily contaminated by secondary invaders. It is necessary only to streak the pus out on the surface of such a medium as Sabouraud's agar and incubate the culture so made at a temperature between that of the room and 37° C. If the pus is contaminated or if the isolation is to be made from sputum the presence of bacteria may interfere with the ready isolation of the fungus. C. E. SMITH recommends the use of a selective medium containing 1% ammonium chloride, 1% sodium acetate, 0.8% tribasic potassium phosphate, and 2% agar. This is autoclaved for 10 minutes at 15 lbs. pressure and 0.04% cupric sulfate is added just before the agar is poured. *C. immitis* makes a slow scanty growth on this medium, but most other organisms are inhibited. The distinguishing characteristics of the fungus will be described in a later paragraph. Any doubt regarding the identity of the fungus isolated can be resolved by injecting its spores intraperitoneally into white mice.

**Therapy:** — Treatment of coccidioidomycosis is not specific and consists principally in supportive therapy. This is usually satisfactory in the case of the primary disease. In progressive coccidioidomycosis there is poor response to therapy. JACOBSON has recommended the use of vaccines and colloidal copper. Penicillin has been tried without success. When lesions are localized, excision or amputation may be indicated.

**Coccidioidin:** — Coccidioidin has been widely used to determine the existence of coccidioidomycosis in an area and to detect a previous exposure or infection in individuals. This sterile, skin-testing antigen is prepared by SMITH (1943) by growing ten strains of *C. immitis* on Bureau of Animal Industry Synthetic Medium (the asparagine broth medium used in making tuberculin) for one to two months. After testing a sample for potency the broth is sterilized by Berkefeld filtration, tested for sterility, and merthiolate to make a final concentration of 1:10,000 is added. Potency and specificity tests are run on persons who have recovered from the mycosis and whose degree of skin sensitivity is known from previous tests, as well as by testing persons who are known to lack sensitization to the fungus. Lots of coccidioidin which are not potent or are not specific are discarded. The undiluted coccidioidin keeps several years, and when diluted 1:100 and kept sterile and refrigerated retains its potency for several months. However it is not recommended that it be used for more than one month after the dilution is made. In testing, 0.1 ml of a 1:100 dilution should be injected intracutaneously. This may cause severe reactions in occasional individuals, and if the person to be tested has had erythema nodosum he should be tested only with a dilution of 1:1000 or 1:10,000.

The test is read in 24 and 48 hours, preferably the latter if only one reading is to be made. Edema only is measured and areas of edema of 5 mm. or more in diameter are considered positive. According to SMITH there is no danger of reactivating arrested lesions, even when the reaction is severe. Persons who have had primary coccidioidomycosis, and particularly those who have had lesions of erythema nodosum or erythema multiforme accompanying the infection, give the most severe reactions. In persons having progressive coccidioidomycosis the reactions are less severe, and in the terminal phases sensitization may entirely disappear.

Coccidioidin, when properly prepared and standardized is generally considered to have a good degree of specificity. The studies of ARONSON *et al.* show that there is no cross reaction with tuberculin. There is cross reaction with *Haplosporangium parvum,* an apparently related fungus which was isolated from a pulmonary mycosis in rodents, but which has not yet been isolated from man. Perhaps 10% of persons living in certain areas of the mid-West and East who have had no known exposure to *Coccidioides* react to coccidioidin. The significance of these non-specific reactions is not known. A skin reaction to coccidioidin usually means past or present infection with *Coccidioides,* but it is possible that there are other fungi which also sensitize to this fungus and whose presence in other areas explains the non-specific reactions. Skin sensitivity once acquired persists for many years, and this must be kept in mind if coccidioidin is being used as a diagnostic agent.

HASSID, BAKER and MCCREADY (1943) isolated an immunologically active polysaccharide from coccidioidin prepared in the standard way on synthetic asparagine medium. This polysaccharide consisted of galacturonic acid, glucose and some unidentified sugar in the approximate ratio of 1 :6.3. A nonprotein nitrogen compound was associated with it. Skin reactions in sensitized individuals were elicited by the polysaccharide but not by a polysaccharide regenerated from an acetyl derivative. Both these sugars were satisfactory for demonstrating precipitins, however.

**Serology:** — COOKE in 1915 demonstrated the presence of precipitins and found no agglutinins in coccidioidal granuloma. DAVIS (1924) also failed to demonstrate agglutinins. He described a complement fixation test, using a suspension of a culture as antigen.

Coccidioidin has been used as an antigen in precipitin and complement fixation tests in coccidioidomycosis, although these serologic tests are not widely used because of the somewhat erratic results obtained. SMITH reported that the tests are rarely positive in mild or inapparent infections. Precipitins are usually present in the more severe primary infections, but not in progressive coccidioidomycosis. In the latter complement fixation may be complete in 1 :256 serum dilution. A fall in precipitins and an increase in complement fixing antibodies may therefore herald dissemination of the infection. The sedimentation rate is increased in coccidioidomycosis and this test may be of value therefore in helping to interpret a positive coccidioidin test.

**Cultures:** — When pus or sputum containing *C. immitis* is planted on agar and incubated at 30° C. growth of the fungus is apparent in three or four days, contrasting with the relatively slow development of most other pathogenic fungi. The appearance is that of a gray or white mold. Although actually the hyphae are rather delicate and of small diameter their tufted and tangled arrangement gives the colony the aspect of a coarse mold. In a colony of a typical strain ten days old there is a central tuft of hyphae surrounded by a narrow zone in which surface growth is sparse. Outside this is a wider zone of cottony tangled mycelium. The color is white at first, becoming gray, and in many strains becoming brown. Some strains develop a lemon yellow pigment. There is little color in the reverse side of an agar culture, although there is an indefinite dark area near the center of the colony in many strains, and an occasional strain produces marked darkening of peptone-containing media. Variation among strains can be readily demonstrated if many strains are compared. Some strains are almost entirely confined to the substratum with very scant development of aerial mycelium. BAKER, MRAK and SMITH (1943) have described some of the variations commonly found.

In many strains the first spores to form in culture appear on specialized branches of the mycelium. These may be simple side branches or they may be more or less elaborately branched. The stalk of one of these branches is of the same diameter as the vegetative hyphae, but it abruptly widens to approximately twice that diameter in its fertile portion. This is particularly apparent in the larger, more complex structures. These specialized fertile hyphae were first described by EMMONS (1942) in many strains of the fungus, and it is probable that they are normally present (*see* Fig. 10). They can be demonstrated only in young cultures soon after the time spores begin to form by carefully teasing out the mycelium in a microscopic mount. When older cultures are examined these specialized hyphae have lost their identity or have disintegrated with the formation of spores, and additional hyphae, poorly or not at all differentiated morphologically have become fertile. One finds then only a tangled mass of vegetative hyphae and fragments of spore chains.

The manner of spore formation is distinctive, although occasional fungi are found as contaminants in which the manner of sporulation is so similar as to be confusing when one is attempting to identify a culture. The fertile hyphae which are destined for spore production are large and engorged, and become divided by frequent septa into barrel-shaped segments. Within these cells the protoplasm condenses so that it occupies one end or, usually, the middle portion of the cell. This results in a chain of spores each separated from the next by a space lacking protoplasm. The original septa dividing the fertile hypha can usually be seen midway between the chlamydospores so formed. When condensation of the protoplasm in two adjacent cells occurs by chance next to the common wall separating them the resulting chlamydospores are of course adjacent to each other, and this breaks the symmetry of the usual arrangement of a chain of spores separated from each other by spaces. The chlamydospores dissociate rather easily when old, but younger spores may remain attached in pairs or short chains. This persistent association of some spores is an important consideration in the interpretation of the structures observed in animals experimentally infected from cultures, as will be noted in a later paragraph. The large number of spores produced in a culture, and the ease with which they are blown about makes the handling of old cultures a dangerous procedure. *C. immitis* should not be grown in plate cultures unless these are destroyed before cultures become old.

**Transition to Parasitic Growth Phase:** — When the chlamydospores of *C. immitis* are injected into an animal they are transformed directly into the parasitic growth phase of the fungus. Each spore rounds up and increases in size until it becomes a spherical cell 30 or 40μ in diameter. When the cell becomes mature (in some cases before it reaches the indicated size, in other cases after becoming much larger) its protoplasm is divided by the formation of cleavage planes into an indeterminate number of endospores (sporangiospores). If the suspension of chlamydospores injected contained short chains of spores these may remain connected during the transformation to the parasitic growth phase and be found as chains of large cells (sporangia) filled with sporangiospores. It is not uncommon to see pairs of such transformed chlamydospores attached to each other by a short tube which represents an empty or dead cell in the original chain of chlamydospores. These bear a superficial resemblance to conjugating pairs of cells but should not be so interpreted. With the rupture of the sporangium and the subsequent escape of the spores the parasitic growth phase of the fungus properly begins, and this will be described in some detail.

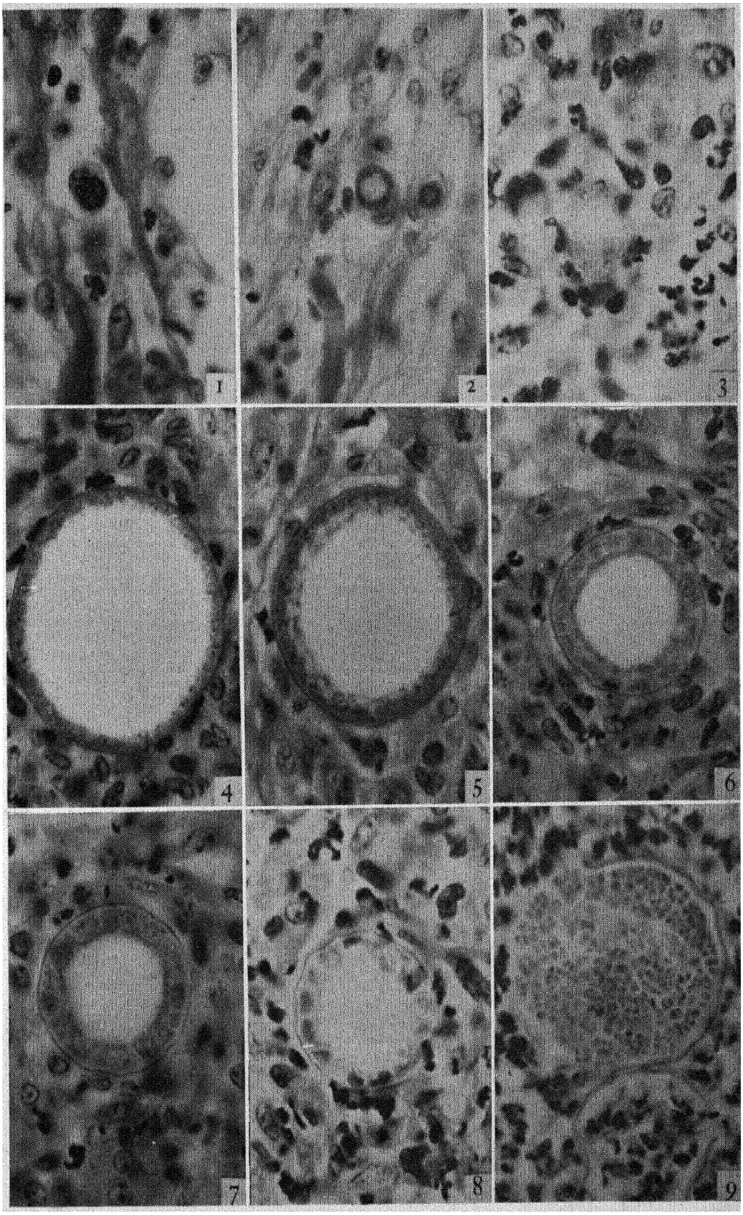
**Parasitic Growth Phase:** — Sporangiospores vary considerably in size but in most cases where subdivision of the protoplasm has progressed normally the liberated spores are 2-3μ in diameter. These may be taken up by any phagocytic cell, perhaps most often by giant cells, or they may remain intercellular. In either case, unless destroyed by the protective mechanism of the host, they proceed to grow. In some tissues, and dependent in part perhaps on strain differences, the growing cell early develops a central vacuole which soon occupies most of the cell and limits the stainable protoplasm to a relatively thin peripheral layer. This layer of protoplasm increases in thickness as the cell matures, and in some cases the central vacuole may be lacking during most of the development of the cell. In the material illustrated (Figs. 1-9), taken from experimentally infected mice, the vacuole was a conspicuous feature except in the mature sporulating cells. As the cell matures small vacuoles appear in the peripheral cytoplasm and these apparently determine the location and direction of the cleavage furrows which initiate sporulation. The first cleavage cuts out large multinucleate masses of protoplasm, and these are progressively subdivided by further cleavage into the ultimate sporangiospores. This process of sporulation seems to be in all essential respects like that found in the Phycomycetes.

When the sporangium becomes filled with spores its outer wall is ruptured, apparently by internal pressures, and the spores escape into the surrounding tissues where they repeat the cycle just outlined. Hyphal formation and budding do not occur during the parasitic growth phase. When material is removed from animal tissue to agar the fungus immediately reverts by the formation of hyphae to the mold or saprophytic growth phase. Spores still within the sporangium, newly liberated spores, partly grown vegetative cells and fully grown sporangia before the initiation of sporulation are capable of this transformation by the production of germination tubes.

The development of sporangia in cultures was demonstrated by LACK (1938) who incubated chlamydospores in glucose broth and partially coagulated egg albumen under reduced oxygen tension. BAKER and MRAK (1941) reported that sporangia ("culture spherules") were produced in ordinary agar cultures of some strains. The nature of these spherule-like bodies found in old cultures of some strains needs further investigation.

Nuclei were demonstrated in material from experimentally infected mice following fixation with Wilson's modification of Bouin's solution, careful dehydration and infiltration, and staining with iron alum hematoxylin (Figs. 3-5). The nuclei are small, with a delicate nuclear membrane, a small amount of chromatin distributed peripherally and a single nucleolus. Unless the process of sporulation is disturbed the ultimate sporangiospores are uninucleate but nuclear division quickly follows their growth.

**Variation:** — During the early studies of coccidioidomycosis two species of *Coccidioides* were described, based on differences in the size of sporangiospores. Subsequent studies showed that spore size is variable and depends upon whether sporulation proceeds normally or whether it is interrupted at some point. The size of the sporangium varies widely also, depending in part upon strain differences, but more importantly upon the host or the tissue in which it is growing. In pus from human cases the size of mature sporangia often averages about 30$\mu$, with occasional sporulating cells much smaller and some larger. In sputum the size is often greater. In mice experimentally



FIGURES 1-9. — *Coccidioides immitis* in experimentally infected guinea pig, showing developmental cycle of the sporangium, the structure and development of nuclei, and the delimitation of spores by progressive cleavage of the cytoplasm. (*From* EMMONS, 1942).

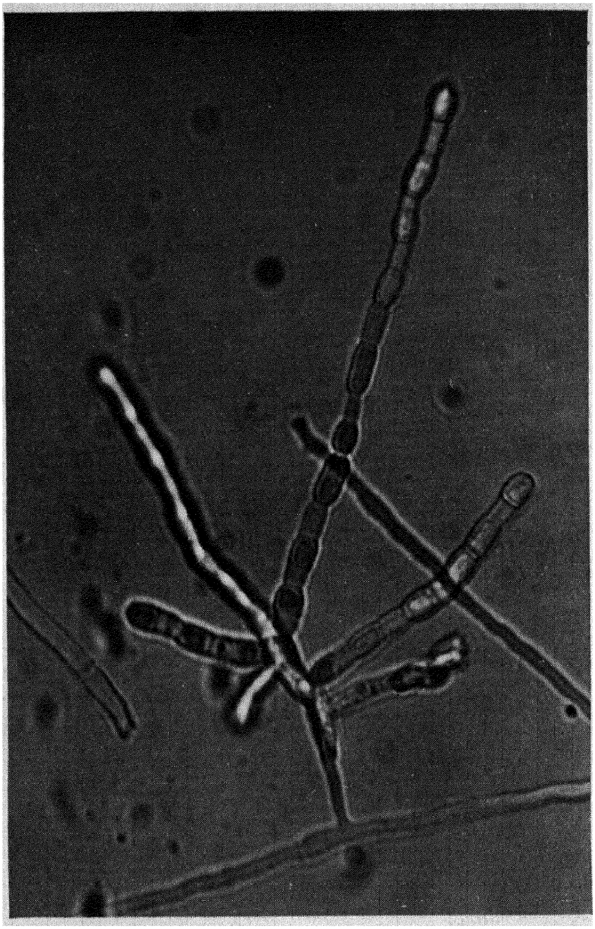


FIGURE 10.—*Coccidioides immitis.* Chains of young chlamydospores borne on specialized branching hyphae from a culture. (*From* EMMONS, 1942.)

infected the sporangia are commonly about 80μ and an occasional sporangium may reach a size of 120μ. In material from man the cells of *C. immitis* may be uniformly filled with protoplasm. In infected mice most strains produce cells with a conspicuous central vacuole and the latter may be so large that only a thin peripheral layer of cytoplasm is seen. It seems probable that large size and the presence of a large vacuole are both indicative of rapid growth in tissue which presents little opposition to the growth of the cell either because of natural susceptibility or because the normal immunological response has been prevented by an overwhelming invasion of the infecting fungus.

*C. immitis* is equally variable in culture. The appearance of a typical strain has already been described. In some strains the typical tufted and tangled aerial mycelium is lacking and the mycelial growth is confined to the substratum where it forms a moist tough layer at and below the surface. Some aerial hyphae may be present near the center, aggregated into strands which resemble coremia. The usual color of the fungus is gray, becoming brownish with age and the formation of spores, but some strains remain pure white while others are yellow. The virulence of strains varies, but is not correlated with any particular colony type. An apparent lessening of virulence is sometimes due to the sparse production of spores with a consequent difficulty in getting a heavy spore suspension for animal inoculation.

**Transmission:**—Although the sporangiospores of *C. immitis* are infectious and it is possible to transmit the disease experimentally with them they do not seem to be important factors in the direct transmission of the disêase. Direct transmission from man to man is not known and probably it is very infrequent. On the contrary the chlamydospores of the fungus are the important disseminating elements and infection occurs usually by the inhalation of these spores. Rarely they are introduced into a wound or abrasion and primary skin lesions result. The spores may be present in windblown dust and it has been generally assumed that the fungus grows in soil during and following the rainy season and that during the dry season the spores mature and are disseminated.

**Coccidioides in Soil:**—*C. immitis* can be isolated from soil by making a suspension of soil in salt solution (30%) in a large cylinder. This is stirred and the coarse material which immediately rises to the top is skimmed off and discarded. The suspension is allowed to stand for an hour, the upper layer is withdrawn, diluted to make approximately a physiological salt solution, centrifuged, and the sediment is planted on agar or injected intraperitoneally into guinea pigs. If cultures are made the selective medium previously mentioned can be used to decrease the growth of other organisms or it can be plated out and suspected colonies can be picked when they appear and transplanted to agar slants until identified. Inoculation into guinea pigs has proved more productive in the author's experience, although undoubtedly many soil samples containing spores are not detected because the amount of inoculum is too small to initiate an infection in the animal.

There are three records of the isolation of *C. immitis* from soil. STEWART and MEYER (1932) isolated it from soil near the bunkhouse of a ranch at Delano, California, where there were men with coccidioidal granuloma. DAVIS and others (1942) isolated it repeatedly in Panoche Valley, California, from soil taken in the immediate vicinity of a burrow from which a rattlesnake had been dug by a group of University students who subsequently developed primary coccidioidomycosis. EMMONS (1943) isolated the fungus from five soil samples taken at random on the desert in the area around San Carlos, Arizona. The occurrence of the fungus in soil is therefore definitely established and supports the concept of air-borne transmission of spores from soil to man. The ecological relationships of this fungus in soil are not known however and there is some doubt whether it normally grows as a saprophyte in soil, as has been usually assumed. It has not been explained, for example, why it is limited in the United States to a comparatively small desert area although it is not fastidious as to mineral nutrients, moisture and temperature in artificial culture in the laboratory. If it had an obligatory association with some desert plant or animal its failure to spread beyond these areas would be more comprehensible. No parasitic or commensal association with a plant has yet been found.

**Animal Reservoir:** — There is some reason to suspect a necessary association with animals. A wide variety of animals are susceptible to experimental inoculation and coccidioidomycosis occurs spontaneously in cattle and sheep grazing in endemic areas. The disease in these animals, however, is usually restricted to the mediastinal nodes. Presumably these animals, as in the case of man are accidentally infected and do not constitute a necessary link in the chain. Several cases of coccidioidomycosis have been reported in the dog but here too the disease is not recognized as common and the dog can hardly be considered as a reservoir.

In studies conducted in Arizona, EMMONS (1942, 1943) observed what may prove to be a true animal reservoir. When small desert rodents were trapped it was found that 80% of the pocket mice (*Perognathus spp.*) examined had a pulmonary mycosis. This was due to one or both of two fungi, *C. immitis* and *Haplosporangium parvum.* The latter is a fungus which appears on immunological and morphological grounds to be related to *C. immitis.* In 124 specimens of *Perognathus, C. immitis* was found in 19 and *H. parvum* in 95. The kangaroo rat (*Dipodomys Merriami*) and the Harris ground squirrel (*Citellus Harrisi*) were also frequently infected. On the contrary, the white-footed mouse (*Peromyscus eremicus*), which was even more abundant than *Perognathus,* and the grasshopper mouse (*Onychomys spp.*) were rarely infected although they were living in burrows adjacent to pocket mice, and the exposure to a fungus living in soil must have been similar. *Peromyscus* is susceptible to a rapidly fatal infection under conditions of experimental infection in the laboratory, but apparently is relatively free of the infection in the field.

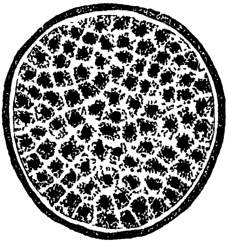
In the animals trapped the observed infection was confined to the lungs, but in some cases the destruction of lung tissue was extensive. It was assumed that the animals eventually succumb but this was not actually proved. Predators and scavengers are common on the desert, and it is assumed that sick and dead rodents are so quickly removed that they were not observed. The infected animals trapped were in many cases pregnant or lactating females and it was apparent that the disease was chronic and progressed so slowly that it did not greatly reduce the rodent population.

It should be noted that the high incidence of infection in rodents awaits confirmation by other observers in other areas, and that proof that the fungus is primarily a parasite of rodents rather than a saprophyte in soil is not yet complete. However, the occurrence of pulmonary coccidioidomycosis in a high percentage of certain species of rodents and its infrequency in other closely associated species which are nevertheless susceptible to laboratory infection, the chronic and slowly progressive form which the mycosis takes in *Perognathus,* and the coincidence between the distribution of these rodents and the endemic area of coccidioidomycosis was interpreted as evidence for a true animal reservoir of coccidioidomycosis. It is suggested that the fungus is present in soil which has been contaminated by infected rodents, and that the distribution of appropriate species limits the distribution of the mycosis.

*Literature Cited:* —

Aronson, J. D., R. M. Saylor and E. I. Parr. Arch. Path. *34:* 31, 1942.
Baker, E. E. and E. M. Mrak. Am. J. Trop. Med. *21:* 589, 1941.
Baker, E. E., E. M. Mrak and C. E. Smith. Farlowia *1:* 199, 1943.
Cox, A. J. and C. E. Smith. Arch. Path. *27:* 717, 1939.
Davis, B. L., R. T. Smith and C. E. Smith. J. A. M. A. *118:* 1182, 1942.
Davis, D. J. Arch. Dermatol. Syphilol. *9:* 577, 1924.
Dickson, E. C. California and Western Med. *47:* 151, 1937.
Dickson, E. C. and M. A. Gifford. Arch. Int. Med. *62:* 853, 1938.
Emmons, C. W. Mycologia *34:* 452, 1942.
Emmons, C. W. Public Health Repts. *58:* 1, 1943.
Emmons, C. W. and L. L. Ashburn. Public Health Repts. *57:* 1715, 1942.
Gifford, M. A. Ann. Rept. Kern County Health Dept. for 1935-36, pp. 22-23, 1936.
Hassid, W. Z., E. E. Baker and R. M. McCready. J. Biol. Chem. *149:* 303, 1943.

LACK, A. R. Proc. Soc. Exptl. Biol. Med. *38:* 907, 1938.
OPHULS, W. and H. C. MOFFITT. Philadelphia Med. J. *5:* 1471, 1900.
OPHULS, W. J. Exptl. Med. *6:* 443, 1905.
RIXFORD, E. and T. C. GILCHRIST. Johns Hopkins Hosp. Rept. *1:* 209, 1896.
SMITH, C. E. Am. J. Public Health. *30:* 600, 1940.
SMITH, C. E. Med. Clin. N. Am. *27:* 790, 1943.
STEWART, R. A. and K. F. MEYER. Proc. Soc. Exptl. Biol. Med. *29:* 937, 1932.



## *Chapter 6*

# RECENT ADVANCES OF THE ITALIAN SCHOOL OF MYCOPATHOLOGY (1941–1945)

*by*

R. Ciferri and P. Redaelli*

**Introduction:** — The difficulties of all kinds inherent in time of war in general slowed research activities greatly in Italy; this was particularly true for the years 1943-45 in Italy. After September 8, 1943, date of the effective dominance of the Germans and Fascists, the paralysis of scientific study, including activity in the field of human mycopathology, was almost complete. This report will cover only the taxonomic work accomplished; clinical cases are omitted.

In the period considered there was published in Italy only one volume related to the fungi pathogenic for man; this was a monograph by Redaelli and Ciferri (1942) on the granulomatous fungi of tropical and subtropical regions. The work was subdivided into a General Part, including: (*1*) Problems of tropical and subtropical climatology in relation to the origin of fungus diseases, (*2*) Pathogenesis and general pathological anatomy of human mycoses in these regions, (*3*) Classification of mycoses and their geographical distribution, and (*4*) Taxonomic mycology, with an analytical key to all of the tribes of the families *Mucedinaceae, Dematiaceae,* and *Stilbaceae,* including 43 new tribes. The authors attempted to present a general picture of the *Hyphales* (excluding the *Tuberculariaceae,* which are scarcely of interest in mycopathology) with analytical keys that may enable students of mycopathology to fit the pathogenic organisms into a general mycological framework. A selected bibliography on the taxonomy of fungi was included.

The second, or Special Part, of the above mentioned volume included: A section on Actinomycosis with a general picture of the taxonomy of the *Actinomycetales,* written by Baldacci (referred to later in this article), which includes the family *Actinomycetaceae,* emend., (2 genera) and the family *Mycobacteriaceae,* emend., divided into the subfamilies *Leptotrichoideae* (5 genera) and *Proactinomycoideae* (5 genera). An analytical key to the genera (*see* Table 1) was appended; 14 fundamental species and a number of doubtful ones were considered. There were also chapters on rhinosporidiosis, coccidioidomycosis, histoplasmosis, and the blastomycoses. The treatment on sporotrichosis included *Sporotrichum Schenckii, S. Councilmani, S. Schenckii* var. *Fioccoi, S. Janselmei, S. Carougeaui,* and *S. anglicum;* it was concluded all mentioned were varieties of *S. Beurmanni.* In the discussion of the mycetoma-maduromycoses the genus *Madurella* was divided into four subgenera: *Eumadurella, Pseudomadurella, Rubromadurella,* and

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* Translated by W. J. Nickerson.

TABLE 1: — *Actinomycetales:* —

*Actinomyces madurae* (Vinc.) Lachn.-Sand, 3 strains with 6 synonyms, and 6 doubtful varieties.
*Actinomyces violaceus* (Rossi-Doria) Gasp., 7 strains with 7 synonyms.
*Actinomyces violaceus* var. *violaceo-ruber* (W. & C.) nov. var.
*Actinomyces violaceus* var. *coelicolor* (Mull.) nov. var.
*Actinomyces coeruleus* n. sp., 4 strains.
*Actinomyces viridis* (Lomb.-Pell.) Dodge, 2 strains.
*Proactinomyces ruber* (Casabo) Baldacci nov. comb., 2 strains with 15 synonyms. and 4 doubtful varieties.
*Proactinomyces pseudomadurae* Baldacci n. sp., 1 strain.
*Proactinomyces polychromogenes* (Vallee) Jens., 3 strains with synonyms.

*Indiella.* This subdivision was based on a previous study by CIFERRI and REDAELLI (9), on *Madurella americana* wherein the identity of the so-called "grani" with the sclerotic fungi was reaffirmed; the probable taxonomic position of *Madurella* in the provisional group of Deuteromycetes labeled "mycelia sterilia" (Agonomycetes), and affinity with the genera *Sclerotium* and *Rhizoctonia* was stated. In particular, this study of *Madurella americana,* and the presence of clamp-connections in cultures, strengthens the assumption of a relationship with the Autobasidiomycete type, *Hypochnus* or *Corticium.* Twelve species of *Madurella* (with key) and some doubtful varieties were considered. On mycetomas caused by *Glenospora* and *Monosporium* a new species, *G. viridobrunnea,* was described that had been isolated in Italy from a case of the foot with black granules.

In the discussion on chromoblastomycosis particular importance was placed on the presence, in certain genera and species, of pycnidia that resemble the ceratopycnidia of the "sooty-molds" (in *Phialophora,* for example), suggesting a relationship with the imperfect *Capnodiales* or, perhaps better, with the *Deuteroperisporiales,* among the *Deuteromeliolae Fumageae;* this view supports the assumption of the identity of *Cadophora* with *Phialophora.* The following new combinations were made: *Phialophora Pedrosoi* and *P. compacta;* a key to the important species involved in chromoblastomycosis was included.

**Coccidioidomycosis in Europe:** — MONTESSORI (18) studying European cases of coccidioidomycosis, compared the two original strains of *Glenospora meteuropea* (neapoletano and balcanico) with *Coccidioides immitis,* confirming that these two strains of *Glenospora* are typical of *C. immitis,* though presumably european in origin. Injection of the strains into animals reproduced coccidioidomycosis, especially in rats. Previous treatments of guinea pigs and rats with *C. immitis* did not, however, modify receptivity for the strains investigated.

An interesting new genus of asporogenous yeasts (*Cystidiella*) was described by MALAN (17). Isolated from mountain soil, the organism is characterized by the production in culture, from its septate mycelia, of accumulations of sessile or pedunculate budding cells which are later fragmented into fusiform particles (eventually refragmented), capable of producing buds or of germinating. The organism is further characterized by a cystidiform body. The genus has been placed in the subfamily *Trichosporonoideae,* being related to a degree to the genus *Trichosporon.* The species *Cystidiella valdensis* is whitish, and incapable of fermenting sugars which it nevertheless utilizes, forming a film on the surface of liquid media.

**Pathogenicity of Mycocandida pseudotropicalis:** — BENEDETTI (3) has reinvestigated intensively *Candida* (*Mycocandida*) *pseudotropicalis* isolated from a case of bronchopneumomycosis. The culture possessed singular physiological and pathological characteristics; it assimilated glucose, starch, and glycogen. Inoculated into various animals the fungus demonstrated a marked pulmonary tropism, giving a picture in the systemic mesenchyme of mycotic reticulo-endotheliosis.

**Pathogenic Species of Geotrichum:** — COLONNELLO (13) has reviewed the clinically important species of *Geotrichum* and their pathological significance in isolates from 30 cases, including various forms of intestinal disease. With 8 strains studied in parallel, all were found referable to *Geotrichum candidum* Link. The only two accepted species of medical importance (*G. candidum* and *G. matalense* (Cast.) Cif.) were found to have practically no pathogenicity or be limited to local action on the tonsils and throat.

**Pathogenic Species of Trichosporon:** — REDAELLI and CIFERRI (19) have studied several freshly isolated strains of *Trichosporon.* They isolated *T. infestans* (Moses and Viana) Cif. & Red. from skin and from gastric juice (in a degraded, pleomorphic form); *T. Beigeli* (Raben.) Vuill. from wood pulp from a paper factory; and *T. Luchettii* n. sp. from three cheeses. *Hemispora coremiformis* Moore has been referred to *T. cutaneum* (Beurm. & Goug.) Ota (= *T. rugosum* etc.). After an analysis of its taxonomy, the genus was thought to belong to the asporogenous yeasts in the subfamily *Trichosporonoideae* (Nannfeldt) Cif. & Red. (1935). Included in the genus *Trichosporon* were the organisms listed in Table 2. An analytical key and complete synonomy was included.

*Aegerita Webberi* Fawc., a parasite in the tropics of Aleyrodes, etc. must be placed, following CIFERRI (11) in a fourth new subgenus (*Chaethaegerita*) by reason of its setose sporodochia. An analogy was seen with the *Rubromadurella* type of *Madurella,* from which point of view *A. Webberi* deserves to be reexamined.

**Pathogenicity of Verticillium for Plants and for Animals:** — VERONA (25) noted that *Verticillium Serrae* (Maffei) v. Beyma (= *Cephalosporium Serrae* Maffei), isolated from a case of ocular mycosis in Italy, belonged to the *Verticillium dahliae* group which causes serious mycotic diseases of the tracheal system in plants. This is then a species which he supposed to possess pathogenicity for man and for higher plants.

TABLE 2: — *Pathogenic Species of Trichosporon:* —

1. *Trichosporon infestans* (Moses & Vianna) Cif. & Red.
2. *Trichosporon Beigeli* (Rabenh.) Vuill.
3. *Trichosporon Luchettii* n. sp.
4. *Trichosporon cutaneum* (Beurm. & Goug.) Ota synonyms:
    *Hemispora coremiformis* Moore
    *Trichosporon rugosum*
5. *Trichosporon giganteum* Behr.
6. *Trichosporon proteolyticum* Negr. & DeVill.-Lastra
7. *Trichosporon Balzeri* Ota
8. *Trichosporon granulosum* Ota
9. *Trichosporon Uffreduzzii* Cif., Crov., & Brun.

CAVALLERO is continuing a revision of the data on immunity and allergy to mycoses. Publications concerning yeasts (4) and dermatophytes (5) have appeared; contributions on the Hyphales and Phycomycetes and the diseases of supposedly mycotic etiology are in course of being printed.

**Studies on the Actinomycetales:** — BALDACCI (1, 2) continued a critical revision of the Actinomycetes, reexamining the organisms listed in Table 1. Appended to the work was a general index to species and varieties; synonomy was brought up to date (203 binomials).

ROSSI (24) studied a case of Simmonds' disease (cryptic hypophysis) with diabetes insipidus based on a circumscribed localization of actinomycotic progression into the hypophysis and diencephalic region in a four-year-old girl. Typical actinomycotic "druse" were found in the anterior lobe of the pituitary and in the tuber cinereum, seats of a granulomatous-purulent process. However, it was not possible to isolate the pathogenic agent.

**Studies on the Caulobacteriales:** — REDAELLI and CIFERRI (21) have studied members of the *Caulobacteriales* using the ammoniacal-silver impregnation method of KIYOSHI-JOKOTA. The technique was used on skin scales and on feces with negative results. With saliva, however, forms of types I, II, and X of the *Caulobacteriales* were found. Types I, IV, and IX were found on certain of the algae, including members of the *Chroococcaceae, Cyanophyceae, Fragillaria* sps., and on *Cladothrix dichotoma.* The forms labeled types I and II are probably *Hyphomicrobium vulgare,* type IV a *Nevskiaceae,* type IX is probably *Blastocaulis,* and type X may be a new family in the same class. Cultures were also made on canal water (Henrici's technique) using a medium of sterile canal water with a 1:1,000,000 solution of $KH_2PO_4$, $MgSO_4$, and $NH_4Cl$ added. The studies are continuing.

**Pathogenic Algae:** — REDAELLI and MARIANI (23) have resumed their study of symbiotic algae of man and of the diseases caused by algae ("algosis," by analogy with mycosis); a general view of recent work in this field was included. The authors considered the genus *Prototheca* (including *P. portoricensis* Cif., Ashf., and Dalmu and the variety *trispora*) in relation to sprue. The new experimental data confirmed a tissue reaction (particularly of the omentum and peritoneum) following experimental inoculations, confirming the former work of CIFERRI and REDAELLI (1940). It was not possible by pretreatments to provoke an allergic state, but occasional, nonspecific chronic intoxications were noticed. The above findings also held for *Prototheca Ciferri* Negroni. The authors reconfirmed the probable relationship of *Blastocystis* with the colorless *Chlorophyceae,* perhaps closely related to the *Protothecaceae,* according to the view of REDAELLI and CIFERRI (1936, '40). Reexamination of the *Oscillatoriae* tended to confirm the probable parasitism of *Oscillatoria enterica* Red. & Silbergleit (1939) as a sustainer of an atrophic colitis in a *Cynocephalus.*

TABLE 3: — *Fungi Isolated from Cadavers: — Relative Occurrence in 50 Isolations from 19 Cadavers.*

| | |
|---|---|
| *Oospora* | 30% |
| *Mortierella* | 18% |
| *Cephalosporium*<br>*Acontium* | 14% |
| *Scopulariopsis*<br>*Penicillium*<br>*Aspergillus* | 10% |
| *Acremonium*<br>*Acremoniella* | 8% |
| *Acrotheca*<br>*Stachybotrys* | 6% |
| *other Hyphales* | 10% |
| *other fungi* | 4% |

**Isolation of Fungi from Cadavers:** — CIFERRI, REDAELLI, and DOMENICI (12) have initiated a study of the fungi that can be isolated from the remains of human cadavers after ten years' burial in the cemetery in Pavia; data were obtained on the tanatological sequence. From 19 cadavers they made 50 isolations, recovering 44 strains of 22 species of fungi. The frequency of occurrence of the organisms was as listed in Table 3. The flora was found to be rather uniform, but was different from that reported by HUNZIKER from Basel. Among the species that were new, were criticized or discussed are those found in Table 4.

An attempt to assemble all the species of fungi isolated by the authors from cadavers during their study indicated: (*1*) in the initial colliquative stages of putrefaction — 10 species, (*2*) isolated from the final stages of putrefaction — 8 species, (*3*) from a study of the preskeletogenous and skeletogenous stages (more completely investigated) — 40 species. The study is continuing to determine the rôle of fungi in the decomposition process.

TABLE 4: — *Some Species Isolated from Cadavers:* —

1. *Acontium album* Morgan
2. *Acontium velatum* Morgan
3. *Acremoniella vaccinii* (Fuckel) Sacc.
4. *Acremonium Bonordeni* Sacc.
5. *Acremonium ossicolum* Cif., Red., & Dom., n. sp.
6. *Acrotheca zignoellae* v. Hohn
7. *Aposphaeriopsis fuscoatra* Died. var. *hemisphaerica,* Cif., Red., & Dom., n. var.
8. *Haplotrichum capitatum* Link
9. *Monilia candida* Sacc.
10. *Oospora cuboidea* Sacc.
11. *Oospora sulphurea* (Preuss) Sacc. & Vogl.
12. *Oospora perpusilla* Sacc.
13. *Oospora penicillioidea* (Riv.) Sacc.
14. *Scopulariopsis minimus* Sart., Huffschm., & Meyer

**Review of Work at the Center for Mycology, Univ. of Pavia:** — REDAELLI and CIFERRI (21) published an account of the first five years' activity (1938-1943) at the center for medical and comparative mycology. The species of fungi studied (177 sps.) of interest in medical and comparative mycopathology were listed, together with the origins of the strains isolated (about 250). A diagnosis of the new species of fungi isolated during the study on cadavers was included.

Among the interesting organisms isolated they noted: (*1*) *Actinomyces melanosporeus* Krainsky from a blood culture of a woman with a fistulous swelling, infiltration, and tumefaction of the knee, (*2*) *Allantospora violacea* Ambrosioni (Riv. Parassitol. 2: 151, 1939) (of the cycle of *Cephalosporium*), (*3*) *Alternaria tenuis* Nees from a dermatiditis verrucosa, (*4*) *Aureobasidium pullulans* (DeBy.) Arn. (= *Dematium pullulans* De Bary = *Pullularia pullulans* Berkh.) isolated from several cases, among which was a chronic metastatic dermatitis of the cheek in a woman in Libya where it was found with *Hormodendron* and *Torulopsis candida,* (*5*) *Cryptococcus psychrophilicus* Nino, a typical *Debaryomyces neoformans* (Sanf.) Red., Cif., & Giord., (*6*) *Cryptococcus meleagris* Bisocci (Nuovo Ercolani 1933), from infectious enterohepatitis in turkeys, which is typical of *Candida* (*Mycotorula*) *albicans.* (*7*) *Cryptomyces pleomorpha* Gruner, a typical *Cephalosporium acremonium* Corda, (*8*) *Geotrichoides rosacea* Maestro (Ann. Oftalm. e Clin. Ocul. *66:* 439-456, 1938), which is a *Fusarium* sp., from a conjunctival mycosis, (*9*) *Candida Guilliermondi,* from a case of "lingua nigra et pilosa, (*10*) *Candida parakrusei* (= *Monilia parapsilosis* Ashford) from a serious diphtherial type pharyngitis, (*11*) *Oospora nivea* (Fuckel) Sacc.

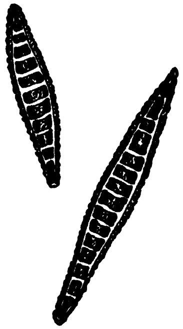
& Vogl. from the abdominal wall and omentum of a dog with purulent, fibrinous peritonitis, (*12*) *Pericystis apis* Massee, inoculated without success into guinea pigs in an attempt to reproduce the histopathological changes of the type caused by *Coccidioides,* (*13*) *Proactinomyces polychromogenes* (Vallee) Jensen from two unknown human cases; *P. Tuber* Casabo from a lesion on the dorsum of the hand and one other case, (*14*) *Sporendonema epizoum* (Corda) Cif. & Red., with critical observations on the taxonomic treatment by Dodge, isolated from salt fish in Canada and from date fruits in Libia, (*15*) *Torulopsis flavescens* (Saito) Lodd. from "yoghurt"; *T. minor* (Poll & Nann.) Lodd., from a case of "lingua nigra et pilosa," (*16*) *Trichosporon Beigeli* (Rabenh.) Vuill., from wood pulp; *T. infestans* (Moses & Vianna) Cif. & Red., from several cases including an epidemic dermatitis caused by metals, etc.

There is, finally, a list of publications, (numbering 98) compiled by Ciferri, Redaelli, and collaborators (1923-27) published before the founding of the Center, and subsequent publications (1938-1943) numbering 65.

### *Literature Cited:—*

1. Baldacci, E. The classification of Actinomycetes. Mycopathologia *3:* 42-43, 1941.
2. Baldacci, E. Contributo alla sistematica degli Attinomiceti: X-XVI. Atti Ist. Bot. Univ. Lab. Critt. Pavia, Series 5, *3:* 139-193, 1944.
3. Benedetti, G. La *Mycocandida pseudotropicalis* (Cast.) Cif. et Red. (Studio sperimentale sulle caratteristiche morfologiche, culturali, biochimiche e patogenetiche del micete). Mycopathologia *3:* 262-281, 1941.
4. Cavallero, C. L'allergia e l'immunità nelle micosi. 2°; Allergia e immunità nelle micosi da lieviti (Blastomicosi). Mycopathologia *3:* 1-41, 1941.
5. Cavallero, C. Id. 3° Allergia e immunità nelle Dermatofizie. *Ibidem 3:* 140-181, 1941.
6. Ciferri, R. A proposito del riferimento di *Emericella varicolor* Berk et Br. ad *Aspergillus* effettuato da Thom e Raper. Mycopathologia *3:* 340-342, 1941.
7. Ciferri, R. Descrizione dei lieviti delle uve, dei mosti e dei vini. In Garoglio, G. P., Trattato di Enologia *2:* 275-309, 1941.
8. Ciferri, R. Il "kamedj" delle palme da dattero (68-70). In Relazione Lab. Critt. Oss. Fitopat. Also Atti Ist Bot. Univ. Lab. Critt. Pavia; Series 5, *1:* 1-53, 1942.
9. Ciferri, R. e P. Redaelli. Sulle affinità e sulla posizione sistematica dei generi *Madurella* e *Indiella.* Mycopathologia *3:* 182-202, 1941.
10. Ciferri, R e P. Redaelli. L'impostazione dello studio di alcuni funghi parassiti o patogeni dell'uomo. (A proposito di una pubblicazione su *Histoplasma*). Mycopathologia *3:* 338-339, 1941.
11. Ciferri, R. e P. Redaelli. Posizione sistematica ed affinità dell '*Aegerita Webberi. Ibidem, 3:* 343-345, 1941.
12. Ciferri, R., P. Redaelli, e F. Domenici. La microflora del cadavere umano. I. Atti Ist. Bot. Univ. Lab. Critt. Pavia, Series 5, *3:* 95-135, 1943.
13. Colonnello, F. Il significato biologico e patologico di alcuni miceti lievitiformi nell'ambiente intestinale dell'uomo. Riv. Clin. Ped. *41* (9), 1943.
14. Colonnello, F. Il *Geotrichum candidum* quale ospite dell'organismo umano. Atti Ist. Bot. Univ. Lab. Critt. Pavia, Series 5, *3:* 195-224, 1944.
15. Domenici, F. La micologia del cadavere umano dopo dieci anni dalla morte. Nota preliminare. Boll. Soc. Med.-Chir. Pavia (6), 1943.

16. Domenici, F. L'indagine micologica nei rispetti della cronologia della morte in caso di decorso abnorme dei comuni fenomeni tanatologici. Arch. Antr. Crim. Pasic. Med. Leg. *62:* 153-156, 1943.
17. Malan, C. E. Cystidiella valdensis, nuovo genere e nuova specie di blastomiceti anascosporei. Mycopathologia *3:* 255-261, 1941.
18. Montessori, P. La granulomatosi coccidioide in Europa. *Ibidem, 3:* 131-139, 1941.
19. Redaelli, P. e R. Ciferri. Nuovi reperti di *Trichosporon* ed osservazioni intorno questo genere. *Ibidem, 3:* 203-217, 1941.
20. Redaelli, P. e R. Ciferri. Le granulomatosi fungine dell'uomo nelle regioni tropicali e subtropicali. In Pollacci, G., Trattato di Micopatologia, *5.* 698 pagg., Firenze (Sansoni Edizioni Scientifiche).
21. Redaelli, P. e R. Ciferri. Primi reperti di Caulobacteriales aquadulcicoli italiani. Mem. Ist. Ital. Idrobiol. M. De Marchi (Atti Conv. Ital. Idrobiol. e Limnol., Milano, 1942, *1:* 107-114, 1942.
22. Redaelli, P. e R. Ciferri. Relazione sul primo quinquennio di attività (1938-1943) del Centro di Micologia Umana e Comparata. Atti. Ist. Bot. Univ. Lab. Critt. Pavia, Series 5, *3:* 1-90, 1943.
23. Redaelli, P. e P. L. Mariani. Notizie generali sulle Alghe parassite dell'uomo e degli animali e studio della loro biologia nei rapporti con gli ospiti. Med. e Biol., *4:* 63-131, 1943.
24. Rossi, G. Morbo di Simmonds da Actinomicosi diencefalo-ipofisaria. Clin. Pediatr. *23:* 1-67, 1941.
25. Verona, O. Un micete patogeno per l'uomo e per le piante. Mycopathol ogia *3:* 50, 1941.



### *Chapter 7*

# THE ACTION OF SULFONAMIDES AND ANTIBIOTIC AGENTS ON THE PATHOGENIC FUNGI

*by*

FREDERICK T. WOLF

## I. SULFONAMIDES

**Introduction:**— The discovery by DOMAGK in 1935 of the chemotherapeutic activity of prontosil (sulfamido chrysoidine) in bacterial infections marked the beginning of great developments in medical bacteriology and chemotherapy. Study of the action of similar compounds—now generally known as the sulfonamides—upon bacteria was soon broadened to include a consideration of the effects of the sulfa drugs upon fungi.

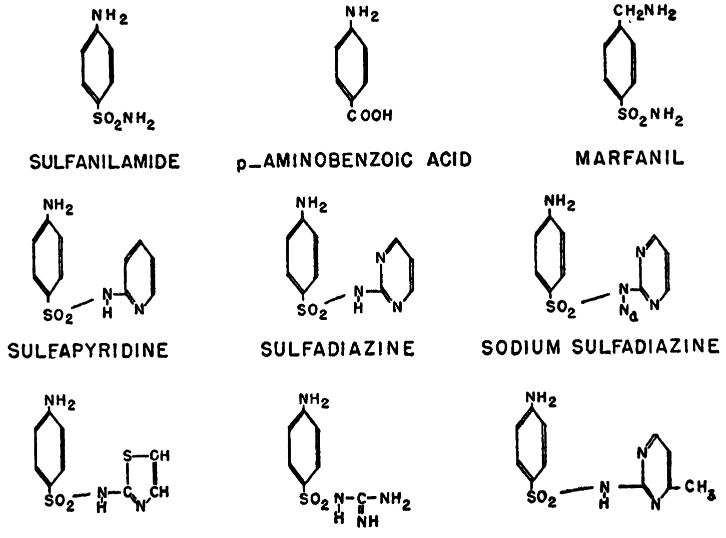
Mycological work with the sulfonamides began with the experiments of FOURNEAU, TREFOUEL, TREFOUEL, NITTI and BOVET (1936), which demonstrated that the active ingredient of prontosil is the substance known as sulfanilamide. Working primarily with *Aspergillus niger,* and to a lesser extent with *A. janselmi, A fumigatus* and *Lichtheimia* (*Mucor*) *italica,* FOURNEAU *et al.* found that the addition of 0.01% sulfanilamide to Raulin's solution resulted in a 24-hour delay in the beginning of growth by the fungi. One part of sulfanilamide per thousand checked growth of *A. niger* for 8-10 days. This organism was able to grow even in media containing 1% sulfanilamide, but only after a delay of two months. It was shown by these experiments that the action of sulfanilamide was fungistatic in character. No evidence of adaptation of the fungi to the drug was noted.

It is perhaps important to realize that these experiments with fungi provided the first proof that sulfanilamide is effective *in vitro* as well as *in vivo,* and antedate all experiments with bacteria on the *in vitro* activity of sulfonamides (LONG and BLISS, 1939).

In a footnote to the paper of FOURNEAU *et al.* (*l.c.*) is to be found the following quotation: "Nous poursuivons actuellement des essais sur l'activité du 1162F (sulfanilamide) dans les mycoses expérimentales."

This study by the workers of the Pasteur Institute is important in that it first clearly established that the active principle of DOMAGK's prontosil is sulfanilamide, that sulfanilamide is effective *in vitro,* that sulfanilamide has fungistatic properties, and it suggested the possibility of using this compound in the treatment of mycotic diseases. The pioneer work of Fourneau *et al.*, although mentioned by Long and Bliss (1939, pp. 87-88), has unfortunately been overlooked by subsequent investigators and therefore has not been given the prominence which it so justly deserves.

**Mechanism of Action:**— Any discussion of the manner by which sulfonamides are effective against bacteria must consider the similarity in chemical structure between the sulfonamides and p-aminobenzoic acid (Fig. 1). Fildes (1940) proposed the hypothesis that antibacterial substances, such as the sulfonamides, act by interfering with an "essential metabolite," *i.e.*, any substance which takes an essential part in the metabolic reactions necessary for bacterial growth. Woods and Fildes (1940) and Woods (1940) found evidence indicating that p-aminobenzoic acid is essential for the growth of streptococci, and demonstrated that this compound very effectively counteracts the inhibition of streptococci by sulfanilamide. They therefore suggested that the inactivation of bacterial cells by sulfanilamide involves a competition for some enzyme between two chemically similar enzyme substrates, the "essential metabolite" (p-aminobenzoic acid) and the inhibitor (sulfanilamide).



SULFANILAMIDE

p-AMINOBENZOIC ACID

MARFANIL

SULFAPYRIDINE

SULFADIAZINE

SODIUM SULFADIAZINE

SULFATHIAZOLE

SULFAGUANIDINE

SULFAMERAZINE

Figure 1. — Structural formulae of certain sulfonamides and p-aminobenzoic acid.

Isolation of p-aminobenzoic acid from natural sources was soon reported by RUBBO and GILLESPIE (1940) and BLANCHARD (1941) from yeast. RUBBO and GILLESPIE (1941) demonstrated, through failure of growth in its absence, the essential nature of p-aminobenzoic acid. SELBIE (1940) first demonstrated the anti-sulfonamide action of p-aminobenzoic acid *in vivo*.

As it pertains to bacteria, the "p-aminobenzoic acid theory" of the action of sulfonamides has been rather generally accepted. In a recent review, WELCH (1945) applies the "lock and key" simile to the mechanism of sulfonamide action. According to this hypothesis, the metabolite is compared to a key, which operates perfectly the "lock" of some enzymatic synthesis. The analog or inhibitor is likened to an imperfect key, which enters the lock but is unable to operate the mechanism, and further, prevents the entrance of the proper key. It was shown by LOCKWOOD and LYNCH (1940) that peptone, a common ingredient of bacteriological and mycological culture media, gives rise to products (not chemically characterized, but other than p-aminobenzoic acid) which likewise interfere with the action of sulfanilamide. For a further consideration of the theories which attempt to explain the anti-bacterial action of the sulfonamides, reference is made to the review of HENRY (1943).

In the case of fungi, DIMOND (1941) has studied the relationship between p-aminobenzoic acid, sulfanilamide, and the growth of *Trichophyton purpureum*. The addition of 1 part per thousand of sulfanilamide to an agar medium upon which *T. purpureum* is inoculated permitted spore germination to occur, but mycelial development was completely inhibited. When, however, p-aminobenzoic acid was added (1 part in 500,000), the inhibition due to sulfanilamide was entirely overcome, and normal growth of the fungus resulted.

Similarly, LANDY and DICKEN (1942) have reported that inhibition of the growth of yeast, brought about by sulfanilamide, sulfapyridine, sulfaguanidine or sulfathiazole in concentrations of 10-25 mg. per cent, can be completely neutralized by p-aminobenzoic acid. When the weight of yeast cells (a function of the number of cells) per unit volume of medium containing a sulfonamide is plotted logarithmically against the concentration of p-aminobenzoic acid, sigmoid curves are obtained.

TATUM and BEADLE (1942) found that the growth of *Neurospora crassa* is inhibited by sulfonamides, and that this inhibition is overcome by p-aminobenzoic acid. This species is normally able to synthesize p-aminobenzoic acid, but as a result of X-ray treatment an "aminobenzoicless" mutant was produced, which was incapable of growth upon media lacking this substance. The respiration of both the normal strain of *N. crassa* and the "aminobenzoicless" mutant has recently been studied by TATUM and GIESE (1946), who found that the respiratory rate is unaffected by sulfanilamide.

Further experiments with p-aminobenzoic acid in relation to the growth of other pathogenic fungi have not been performed. The behavior of other pathogenic fungi requiring p-aminobenzoic acid may be no different from that reported for yeast and for *T. purpureum.* In general, therefore, the inhibition of fungus growth by the sulfonamides may, in all probability, be regarded as follows: (*1*) the fungus, in the course of its normal metabolism, requires p-aminobenzoic acid, (*2*) p-aminobenzoic acid is very similar in chemical structure to the sulfonamides, (*3*) the sulfonamide is absorbed and saturates an enzyme system of the fungus, and (*4*) some of the syntheses required for growth of the fungus cannot then be performed.

LWOFF, NITTI, TREFOUEL and HAMON (1941) have shown that the antifungal activity of a sulfonamide is greatly influenced by the acidity of the medium. Using *Aspergillus niger* grown in Czapek's solution, the concentration of sulfanilamide which would produce a delay in growth of the fungus for 24 hours more than control cultures was determined at various *p*H levels. At *p*H 7.1, sulfanilamide 1:200,000 produced such a delay in the growth of *A. niger;* at *p*H 5.8, only 1:20,000 sulfanilamide was required; at *p*H 4.6, 1:10,000; and at *p*H 3.7, 1:5,000. Thus the activity of sulfanilamide against *A. niger* decreased as the medium became more acid.

**In Vitro Experiments:**— Studies of the action of sulfonamides on pathogenic fungi, *in vitro,* although admittedly inadequate in several respects, nevertheless constitute a rational approach to the problem of successful chemotherapy of mycotic infections. It has already been noted that FOURNEAU, TREFOUEL, TREFOUEL, NITTI and BOVET (1936) performed the first experiments concerning the *in vitro* effects of sulfonamides on fungi. The addition of sulfanilamide to a medium in which *Aspergillus niger* was grown resulted in a marked inhibition of growth. 0.01% sulfanilamide inhibited growth completely for one day, 0.1% sulfanilamide delayed growth for 8-10 days, and a 1% concentration of the drug checked growth of *A. niger* for as long as two months. Essentially similar results were noted with *A. janselmi, A. fumigatus,* and *Lichtheimia (Mucor) italica.* The action of sulfanilamide was therefore primarily one of fungistasis.

The first observations concerning the effect of a sulfonamide upon a pathogenic fungus were made by DIMOND (*l.c.*) who reported that 0.1% sulfanilamide checked growth of *Trichophyton purpureum,* and that this inhibitory action could be overcome by antagonism with p-aminobenzoic acid.

The effects of sulfanilamide, sulfathiazole and sulfadiazine upon two strains of *Actinomyces bovis (hominis)* were studied by CUTTING and GEBHARDT (1941). The organisms were grown on Krainsky's glucose agar and on a thioglycollate medium under both aerobic and anaerobic conditions. Sulfonamides were added to the media in quantities equivalent to 10, 50 and 100 mg. per cent. Slight inhibition was obtained with 10 mg. per cent of sulfanilamide, while 50 or 100 mg. per cent checked growth more or less completely. In similar concentrations, both sulfathiazole and sulfadiazine were more effective than sulfanilamide.

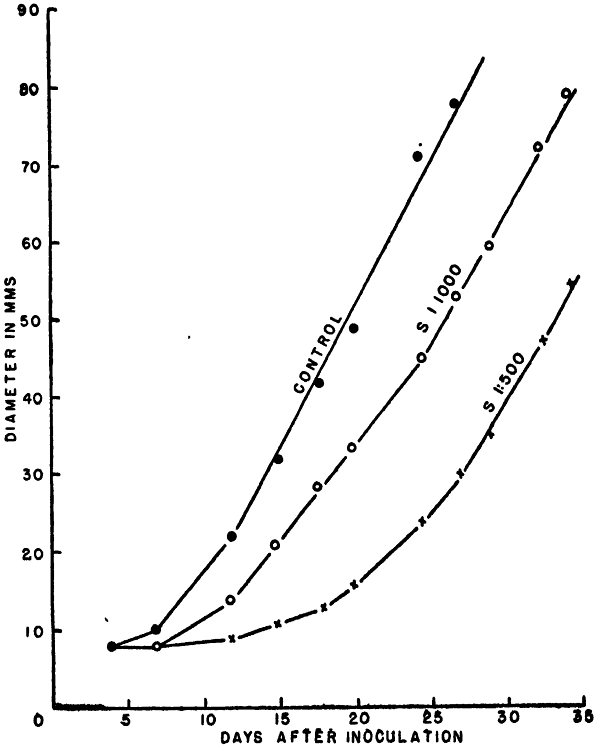


Figure 2. — Growth of *Trichophyton purpureum* on a medium containing various amounts of sulfanilamide (*after* Dimond and Thompson, 1942).

Simultaneously, but independently of other workers, Lewis and Hopper (1941) made a thorough study of the action of six sulfonamides upon *Trichophyton gypseum* and *Candida albicans*. The sulfonamides available to them were sulfanilamide, sodium sulfapyridine, sulfathiazole, sodium sulfathiazole, sulfadiazine and sodium sulfadiazine. Concentrations of 1%, 5% and 10% were employed, in spite of insolubility of the compounds, which resulted in crystallization in all instances at the 5% and 10% concentrations, and in certain instances at the 1% level.

None of the compounds proved effective in causing any decrease in the growth of *Candida albicans*. Each of the compounds exhibited some growth-inhibitory action upon *Trichophyton gypseum,* but sulfanilamide was the most effective compound. With 1% sulfanilamide, three weeks were required for minute colonies to appear.

These results were confirmed and extended by the work of DIMOND and THOMPSON (1942) upon *Trichophyton gypseum* and *T. purpureum.* Experiments were performed in which spores were suspended in 0.1% solutions of sulfanilamide, sulfapyridine, sulfathiazole, sulfaguanidine or sodium sulfadiazine for various periods of time, after which they were washed and inoculated upon an agar medium. Such cultures grew normally after the removal of the drug, and no signs of injury as a result of this treatment were apparent. The action of the sulfonamides was thus again shown to be a fungistatic rather than a fungicidal one.

Further experiments, with agar media containing sulfonamides in concentrations ranging from 1:500 to 1:10,000, demonstrated that sulfanilamide was far more effective than sulfapyridine, sulfathiazole, sulfaguanidine or sodium sulfadiazine in inhibiting growth of *T. gypseum* and *T. purpureum.*

Measurements of the growth rate of *T. purpureum* on honey-peptone agar containing varying amounts of sulfanilamide showed that the fungistatic action of sulfanilamide was to be interpreted as an extension of the lag phase of the growth curve (Fig. 2). In other words, sulfanilamide will delay the beginning of growth of fungi for a time, but has absolutely no influence upon the rate of growth, once growth has begun. It was further shown by DIMOND and THOMPSON (*l.c.*) that the fungistatic action of sulfanilamide was much more pronounced on a peptone-free medium, since peptone gives rise to anti-sulfonamide factors.

NOOJIN and CALLAWAY (1943) have determined the action of seven sulfonamides upon *Blastomyces dermatitidis* grown on Sabouraud's agar. Each drug was tested in concentrations ranging from 50 to 250 mg. per cent. The results are presented in the following table:

TABLE 1: *Action of sulfonamides upon the growth of Blastomyces dermatitidis* (NOOJIN and CALLAWAY, 1943) :—

| | *Concentration, mg. per cent* | | | | | | |
|---|---|---|---|---|---|---|---|
| SULFONAMIDE | 50 | 75 | 125 | 150 | 175 | 200 | 250 |
| Sulfadiazine | ± | ± | 0 | 0 | 0 | 0 | 0 |
| Sulfanilamide | + | + | + | ± | 0 | 0 | 0 |
| Sulfapyridine | + | + | + | + | ± | ± | 0 |
| Na sulfapyridine | + | + | + | + | + | + | 0 |
| Sulfathiazole | + | + | + | + | + | + | + |
| Na sulfathiazole | + | + | + | + | + | + | + |
| Sulfaguanidine | + | + | + | + | + | + | + |

Thus, sulfathiazole, sodium sulfathiazole and sulfaguanidine were completely without fungistatic activity against *B. dermatitidis* in all levels tested, sulfapyridine and sodium sulfapyridine were slightly fungistatic in high concentrations, and sulfadiazine and sulfanilamide were the most effective agents against *B. dermatitidis.* Since 10 mg. per cent represents the maximal sulfonamide blood level which can be maintained for considerable periods and tolerated by the patient, clinical use of sulfonamides in the treatment of blastomycosis is necessarily limited to local applications to cutaneous lesions.

NOOJIN and CALLAWAY (1944) subsequently studied the action of sulfonamides upon the growth of *Sporotrichum schenckii.* The sulfonamides used were the same as in their previous work with *Blastomyces dermatitidis,* but with the addition of two new derivatives, sulfapyrazine and sodium sulfapyrazine. Sabouraud's agar was used, containing the drugs in concentrations of 50-1,000 mg. per cent. The results are presented in the following table:

TABLE 2: *Action of sulfonamides upon the growth of Sporotrichum schenckii* (NOOJIN and CALLAWAY, 1944) :—

| | *Concentration, mg. per cent* | | | |
|---|---|---|---|---|
| SULFONAMIDE | 50 | 250 | 500 | 1,000 |
| Sulfanilamide | +++ | +++ | ++ | + |
| Na sulfapyridine | +++ | +++ | ++ | + |
| Na sulfapyrazine | +++ | +++ | ++ | ++ |
| Na sulfathiazole | +++ | +++ | +++ | ++ |
| Sulfapyridine | +++ | +++ | +++ | ++ |
| Sulfadiazine | +++ | +++ | +++ | +++ |
| Sulfathiazole | +++ | +++ | +++ | +++ |
| Sulfaguanidine | +++ | +++ | +++ | +++ |
| Sulfapyrazine | +++ | +++ | +++ | +++ |

Sulfanilamide and sodium sulfapyridine were the most effective compounds against *Sporotrichum schenckii,* but again, as with *B. dermatitidis,* high concentrations of the compounds were required to produce a growth-inhibitory action. Topical application of sulfanilamide or sodium sulfapyridine to the granulomatous lesions of cutaneous sporotrichosis was recommended by NOOJIN and CALLAWAY as a result of these experiments.

SENTURIA and WOLF (1945) studied the action of sulfonamides applied to cultures of fungi isolated from cases of otomycosis. The growth of *Aspergillus fumigatus, A. niger, A. glaucus, A. sydowi,* and *Mucor corymbifer* upon plates of Sabouraud's agar was markedly inhibited by sulfanilamide, when the quantity of the drug approximated 20-30 mg. per culture. Similar amounts of sulfathiazole, sulfadiazine, sulfaguanidine or sulfamerazine were ineffective. *Candida albicans* was unaffected by any of these sulfonamides, confirming the finding of LEWIS and HOPPER (1941). It is interesting to compare these results with those of FOURNEAU *et al.* (*l.c.*). *Aspergillus niger* and *A. fumigatus* were employed as test organisms in each case, with identical results upon treatment with sulfanilamide.

KEENEY, AJELLO and LANKFORD (1944) have investigated the action of sodium salts of sulfathiazole, sulfadiazine and sulfamerazine upon many of the common pathogenic fungi and *Actinomyces bovis*, taking into account the action of sulfonamide antagonists. The sodium salts of the sulfonamides were selected for study because of their greater solubility and theoretically greater promise of successful systemic use. The fungi studied were *Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum, Microsporum audouini, M. felineum, Candida albicans, Cryptococcus neoformans, Blastomyces dermatitidis, Sporotrichum schenckii, Coccidioides immitis, Phialophora pedrosoi, Histoplasma capsulatum* and *Actinomyces bovis.* All organisms were grown in dextrose broth except *A. bovis,* for which a thioglycollate medium was used.

The quantity of each of the sulfonamides necessary to neutralize their respective antagonists present in the media was determined by adding sulfonamides in different quantities to the media, inoculating with *Staphylococcus aureus,* and determining, for each medium and each sulfonamide, the lowest sulfonamide concentration which would just permit apparent growth. These concentrations varied from 100 to 800 mg. per cent.

Tubes of dextrose broth containing 50-1,000 mg. per cent of the various sulfonamides, in excess of the amounts required to neutralize the sulfonamide antagonists, were then inoculated with fungi, incubated, and examined after three weeks. The results are presented in the following table:

TABLE 3: *Action of sulfonamides upon the growth of pathogenic fungi* (KEENEY, AJELLO and LANKFORD, 1944):—

| | | *Na sulfathiazole mg. %* | | *Na sulfadiazine mg. %* | | *Na sulfamerazine mg. %* | |
|---|---|---|---|---|---|---|---|
| ORGANISM | Control | 100 | 1,000 | 100 | 1,000 | 100 | 1,000 |
| *T. mentagrophytes* | ++++ | ++++ | + | +++ | + | ++++ | + |
| *T. rubrum* ....... | ++++ | ++++ | + | ++ | + | ++++ | ++ |
| *E. floccosum* ..... | +++ | +++ | +++ | +++ | + | ++ | + |
| *M. audouini* ..... | + | + | — | + | — | + | — |
| *M. felineum* ..... | ++++ | ++++ | ++ | ++++ | ++++ | ++++ | ++++ |
| *C. neoformans* ... | ++++ | +++ | + | ++ | — | +++ | + |
| *C. albicans* ...... | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| *B. dermatitidis* ... | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *C. immitis* ....... | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *S. schenckii* ..... | ++++ | ++ | ++ | ++++ | ++ | +++ | ++ |
| *P. pedrosoi* ...... | +++ | — | — | + | — | — | — |
| *H. capsulatum* ... | + | — | — | — | — | — | — |

Little or no significant fungistatic activity was obtained except with *Microsporum audouini, C. neoformans, P. pedrosoi* and *H. capsulatum.* Inhibition of growth of *M. audouini* and *C. neoformans* largely disappeared, however, when the organisms were grown on agar media containing whole blood or serum. The clinical trial of sodium sulfamerazine in the treatment of chromoblastomycosis and of sodium sulfathiazole in the treatment of histoplasmosis was recommended as a result of these experiments.

It was further shown by KEENEY, AJELLO and LANKFORD (*l.c.*) that these sulfonamides were fungicidal when a sufficiently high concentration was employed. With *Actinomyces bovis* in thioglycollate media, concentrations of 500 or 1,000 mg. per cent of sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine produced both fungistatic and fungicidal effects.

WOLF (1945) studied the action of sulfanilamide, sulfathiazole, sulfadiazine and sulfaguanidine upon the common pathogenic fungi. These were *Candida albicans, C. krusei, C. tropicalis, C. parakrusei, C. pseudotropicalis, Cryptococcus neoformans, Epidermophyton floccosum, Trichophyton mentagrophytes, T. rubrum, Monosporium apiospermum, Hormodendrum pedrosoi, H. compactum, Phialophora verrucosa, Microsporum canis, M. gypseum* and *Sporotrichum schenckii.*

Sulfanilamide was very markedly fungistatic to all of the dermatophytes (*Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum, Microsporum canis, M. gypseum*) and to *Sporotrichum schenckii.* Sulfanilamide had no pronounced activity against any of the remaining fungi tested, and sulfathiazole, sulfadiazine and sulfaguanidine were not effective against any of the pathogens.

The results of the various investigators as to the susceptibility of pathogenic fungi to sulfonamides have been assembled in Table 4.

The basis for the great antifungal activity *in vitro* of sulfanilamide, noted by FOURNEAU *et al.* (1936), LEWIS and HOPPER (1941), DIMOND (1941), DIMOND and THOMPSON (1942), NOOJIN and CALLAWAY (1943, 1944), SENTURIA and WOLF (1945), and WOLF (1945) has not been adequately explained, but it seems evident that sulfanilamide has a greater antimycotic activity, against many of the pathogenic fungi, than does any other sulfonamide.

One possible theoretical criticism may be made of studies which attempt to compare the action of the sulfonamides *in vitro* with that of their sodium salts. Owing to the high *p*H of solutions of the sodium derivatives of the sulfonamides (8.2 in the media used by LEWIS and HOPPER, 1941) it may well be that a portion of the fungistatic activity of these compounds is a direct effect of *p*H rather than a specific chemical action upon the fungus by the compound concerned. This objection was recognized both by LEWIS and HOPPER (1941) and

Table 4: *Summary of the sulfonamides effective in vitro against pathogenic fungi:—*

| ORGANISM | *Sulfonamides effective* | *Investigator* |
|---|---|---|
| *Aspergillus fumigatus* | sulfanilamide | Fourneau *et al.* (1936),<br>Senturia and Wolf (1945). |
| *A. glaucus* | sulfanilamide | |
| *A. janselmi* | sulfanilamide | |
| *A. niger* | sulfanilamide | |
| *A. sydowi* | sulfanilamide | |
| *Mucor (Lichtheimia) italica* | sulfanilamide | |
| *M. corymbifer* | sulfanilamide | |
| *Trichophyton gypseum (mentagrophytes)* | sulfanilamide | Dimond (1941), Lewis and Hopper (1941),<br>Dimond and Thompson (1942), Wolf (1945). |
| *T. purpureum* | sulfanilamide | |
| *T. rubrum* | sulfanilamide | |
| *Epidermophyton floccosum* | sulfanilamide | Keeney *et al.* (1944), Wolf (1945). |
| *Microsporum felineum* | ........ | |
| *M. canis* | sulfanilamide | |
| *M. gypseum* | sulfanilamide | |
| *M. audouini* | Na sulfathiazole<br>Na sulfadiazine<br>Na sulfamerazine | |
| *Blastomyces dermatitidis* | sulfadiazine<br>sulfanilamide | Noojin and Callaway (1943). |
| *Sporotrichum schenckii* | sulfanilamide<br>Na sulfapyridine | Noojin and Callaway (1944), Wolf (1945). |
| *Hormodendrum (Phialophora) pedrosoi* | Na sulfathiazole<br>Na sulfadiazine<br>Na sulfamerazine | Keeney *et al.* (1944), Wolf (1945). |
| *H. compactum* | ........ | |
| *Phialophora verrucosa* | ........ | |
| *Monosporium apiospermum* | ........ | |
| *Histoplasma capsulatum* | Na sulfathiazole<br>Na sulfadiazine<br>Na sulfamerazine | |
| *Coccidioides immitis* | ........ | |
| *Cryptococcus neoformans* | Na sulfadiazine | |
| *Candida (Monilia) albicans* | ........ | Lewis and Hopper (1941),<br>Senturia and Wolf (1945),<br>Wolf (1945). |
| *Candida krusei* | ........ | Wolf (1945). |
| *C. tropicalis* | ........ | |
| *C. parakrusei* | ........ | |
| *C. pseudotropicalis* | ........ | |
| *Actinomyces bovis (hominis)* | sulfanilamide<br>sulfathiazole<br>sulfadiazine<br>Na sulfathiazole<br>Na sulfadiazine<br>Na sulfamerazine | Cutting and Gebhardt (1941),<br>Keeney *et al.* (1944). |

Noojin and Callaway (1944). It is probable that studies which now consider optimal media and optimal temperatures for the growth of pathogenic fungi, and the question of sulfonamide antagonists present in the media, must also consider optimal *p*H, when drugs of differing *p*H are to be compared *in vitro.*

No data are yet available concerning the action of Marfanil (homosulfanilamide) upon fungi. The study of the action of this drug, as well as that of other newer sulfonamide derivatives, upon fungi must await future laboratory experimentation.

**Animal Experimental Studies:**— From the time of discovery of a new drug until it is placed into general use, there are usually several important steps which intervene including: (*1*) "test tube" experiments to determine activity *in vitro,* (*2*) more stringent *in vitro* studies, in which blood or serum is added to the system, to determine the degree of interference under these conditions, (*3*) animal experimental studies, to test activity of the compound *in vivo* and to determine its toxicity to body tissues, and (*4*) clinical trials, in the treatment of selected human cases.

It has been mentioned that Fourneau *et al.* (*l.c.*) visualized the possibility of using the sulfonamides in the therapy of experimental fungus diseases in animals. In view of the numerous clinical trials which have been made of sulfonamides in cases of mycotic infections, it is surprising that more attention has not been devoted to animal experimental studies.

Dosa (1941) investigated the effect of a sulfonamide upon experimental torulosis, blastomycosis and actinomycosis in animals. Rats were chosen as the experimental animal, and the sulfonamide used was the proprietary preparation "Neosanamid" (p-(p-acetylaminobenzolsulfon) iminobenzolsulfonamid), given orally. Upon inoculation, the animals were divided into four groups. Group I included animals to which the drug was administered immediately upon inoculation, and thereafter in decreasing dosages for 12 days. The animals in group II were treated similarly, but the dosage was only one half of that for group I. The drug was administered to group III animals only upon the appearance of disease symptoms. The control animals, group IV, were not treated.

Mortality in the control group was 100%, 10-12 days being the duration of life in animals with torulosis or blastomycosis, and 21-25 days in the case of animals inoculated with *Actinomyces.* Post mortem pathologic examination disclosed numerous lesions and the characteristic organisms within the tissues. In groups I-III, the mortality rates were 5%, 40%, and 20% respectively. No instance of sickness due to administration of the sulfonamide was noted, and the fact that the symptomatic animals recovered in large measure upon treatment clearly indicates possible success in the use of sulfonamide chemotherapy in the systemic mycoses.

JONES and KLINCK (1945) have recently reported the results of studies upon experimental torulosis in mice, in which sulfadiazine was found to be ineffective.

**Clinical Results:**— A detailed discussion of the treatment of mycotic infections by use of sulfonamides is beyond the scope of this work, and may be found in medical and clinical texts. A few general remarks concerning the relation between the results of laboratory research on the pathogenic fungi and their practical application may not, however, be inappropriate. Likewise, it is here our intention to refer briefly to the more important clinical papers, and to state an opinion as to the probable value of sulfonamides in each of the various mycotic diseases.

The basis of sulfonamide treatment of fungus diseases has been largely empirical. In many instances, clinical results have antedated *in vitro* studies, animal experimental studies have been largely nonexistent, and in a few cases, reports of successful clinical cures are not in agreement with the results of laboratory *in vitro* studies. For some diseases, the number of cases upon which reports of cures are based appears to be too small to justify any definite conclusion. Reported failures, in some instances, may be due to the fact that sulfonamide therapy was instituted only *in extremis,* when more accepted means of therapy had failed.

Of far greater importance, however, is the fact that successful therapy of a fungus disease implies far more than merely killing the causative organism. The pathologic changes which occur in the tissues are largely manifestations of reactions of the tissues to the fungus, and generally the question of physical damage due to the mere presence of a foreign organism is of relatively minor importance. Hence the emphasis on autogenous vaccines and other measures whose primary purpose is one of desensitization.

Further, it is to be recognized that successful therapy usually is not attained through any single agent, no matter what its properties, except in conjunction with surgery, desensitization procedures, measures for control of secondary infection, and others when indicated.

Spectacular results have been obtained by means of sulfonamides in the treatment of actinomycosis. Abdominal, thoracic and cervicofacial types of this disease have been rapidly and completely cured. Sulfanilamide or sulfapyridine has been the drug most often employed. Following the pioneer work of WALKER (1938), a great number of case reports have appeared. These include those of MILLER and FELL (1939), HALL (1939), DORLING and ECKHOFF (1940), OGILVIE (1940), CHRISTOPHER and KARABIN (1940), WILKINSON (1941), DOBSON, HOLMAN and CUTTING (1941), MITCHELL (1942), McCLOY (1943), LYONS, OWEN and AYERS (1943), LADD and BILL (1943), HOLLENBECK and TURNOFF (1943), BILLINGTON (1944), and BENBOW, SMITH and GRIMSON (1944). The results obtained upon sulfonamide therapy of actinomycosis, when employed in conjunction with surgical measures, have been sufficiently good to justify the use of sulfonamides as a standard method of treatment of this infection.

The successful treatment of a case of maduramycosis (Madura foot), in which the causative organism was an actinomycete, by the oral administration of sulfanilamide was reported by DIXON (1941).

REEVES, BUTT and HAMMACK (1941) and MARSHALL and TEED (1942) reported recovery, following sulfonamide therapy, in cases of torulosis (cryptococcosis, European blastomycosis) of the meninges and central nervous system, in which the prognosis is always extremely poor.

According to NAVARRO-MARTIN (1940), sporotrichosis also responds to sulfonamide therapy.

SCHROEDER (1940) and ALBERT (1943) report the cure of blastomycosis as a result of sulfonamide therapy. Other instances of successful treatment of blastomycosis are to be found in some of the more obscure South American journals. In the case of South American blastomycosis caused by *Blastomyces brasiliensis,* IRIARTE (1945) reports successful therapy in two cases, in one of which sulfapyridine was used, and sulfathiazole in the other instance.

PFALZGRAF (1941) employed a prontosil solution in the treatment of epidermophytosis. The results of more recent large scale tests, however, would appear to show that sulfonamides may do a great deal of harm in "athletes' foot" and allied conditions, and are definitely contra-indicated.

VAN BREE (1941) reported marked improvement in a case of generalized moniliasis, an observation which is difficult to understand in the light of *in vitro* studies. WESSLER and BROWNE (1945) obtained no beneficial results in a case of *Candida albicans* infection of the blood stream upon sulfonamide treatment.

Various bronchopulmonary mycoses, in which *Blastomyces, Aspergillus, Penicillium, Mucor, Sporotrichum* or *Actinomyces* was involved, respond to sulfonamide therapy in the experience of DE ALMEIDA and LACAZ (1942).

MOORE and JORSTAD (1943) report the failure of sulfonamides in cases of histoplasmosis, while ALONSO and FREIJO (1944) obtained recovery following sulfadiazine therapy.

GOLDSTEIN and McDONALD (1944) obtained no beneficial results with sulfonamides in a case of primary pulmonary coccidioidomycosis.

Otomycosis or external otitis, an infection of the external ear canal caused by various fungi including species of *Aspergillus* and *Mucor,* while by no means a serious infection in comparison with other mycoses, has been a problem of some importance to military and naval personnel in tropical and sub-tropical areas. SENTURIA (1944) of the Army Air Forces reported success in treatment of this disorder by local applications of sulfanilamide, sulfathiazole and zinc peroxide. This information was made available to naval medical officers through an anonymous article in the Bumed News Letter (1944). More recently SIMON (1945) of the Navy has experienced good results with a combination of systemic sulfathiazole and local sulfanilamide therapy.

In conclusion, it would appear that laboratory studies of the pathogenic fungi have played an important part in the development of methods of therapy, which in some fungus diseases are quite successful, but in other cases show the need for further fundamental laboratory and clinical experiments.

*Literature Cited:—*

ALBERT, M. Brit. J. Dermat. *55:* 294, 1943.
DE ALMEIDA, F. and C. DA SILVA LACAZ. Comp. Melhoramentos de São Paulo. 1942.
ALONSO, J. M. and J. C. FREIJO. Arch. Urug. Med., Cir., y Especialidad. *24:* 193, 1944.
(Anonymous). Bumed News Letter *3:* (11), 12, 1944.
BENBOW, E. P. JR., D. T. SMITH and K. S. GRIMSON. Am. Rev. Tuberc. *49:* 395, 1944.
BILLINGTON, R. W. Brit. Med. J. *1:* 326, 1944.
BLANCHARD, K. C. J. Biol. Chem. *140:* 919, 1941.
CHRISTOPHER, F. and J. E. KARABIN. Am. J. Surg. *50:* 371, 1940.
CUTTING, W. C. and L. P. GEBHARDT. Science *94:* 568, 1941.
DIMOND, N. S. Science *94:* 420, 1941.
DIMOND, N. S. and K. W. THOMPSON. J. Invest. Dermat. *5:* 397, 1942.
DIXON, J. M. Virginia M. Monthly *68:* 281, 1941.
DOBSON, L., E. HOLMAN and W. CUTTING. J. A. M. A. *116:* 272, 1941.
DORLING, G. C. and N. L. ECKHOFF. Lancet *2:* 707, 1940.
DOSA, A. Acta Dermato-Venereologica *22:* 315, 1941.
FILDES, P. Lancet *1:* 955, 1940.
FOURNEAU, E., J. TREFOUEL, MME. J. TREFOUEL, F. NITTI and D. BOVET. C. R. Soc. Biol. *122:* 652, 1936.
GOLDSTEIN, D. M. and J. B. MCDONALD. J. A. M. A. *124:* 557, 1944.
HALL, W. E. B. J. A. M. A. *112:* 2190, 1939.
HENRY, R. J. Bact. Revs. *7:* 175, 1943.
HOLLENBECK, W. F. and D. TURNOFF. J. A. M. A. *123:* 1115, 1943.
IRIARTE, D. R. Biol. Lab. Clin. Luis Razetti *6:* 277, 1945.
JONES, S. H. and G. H. KLINCK, JR. Ann. Internal Med. *22:* 736, 1945.
KEENEY, E. L., L. AJELLO, and E. LANKFORD. Bull. Johns Hopkins Hosp. *75:* 393, 1944.
LADD, W. E. and A. H. BILL, JR. New England J. Med. *229:* 748, 1943.
LANDY, M. and D. M. DICKEN. Nature *149:* 244, 1942.
LEWIS, G. M. and M. E. HOPPER. Arch. Dermatol. Syphilol. *44:* 1101, 1941.
LOCKWOOD, J. S. and H. M. LYNCH. J. A. M. A. *114:* 939, 1940.
LONG, P. H. and E. A. BLISS. The clinical and experimental use of sulfanilamide, sulfapyridine and allied compounds. 319 pp., Macmillan, New York, 1939.
LWOFF, A., F. NITTI, MME. J. TREFOUEL and V. HAMON. Ann. Inst. Pasteur *67:* 19, 1941.
LYONS, C., C. R. OWEN and W. B. AYERS. Surgery 14: 99, 1943.
MARSHALL, M. and R. W. TEED. J. A. M. A. *120:* 527, 1942.
MCCLOY, A. Brit. Med. J. *2:* 106, 1943.

MILLER, E. M. and E. H. FELL. J. A. M. A. *112:* 731, 1939.
MITCHELL, H. S. Canadian Med. Assn. J. *46:* 584, 1942.
MOORE, M. and L. H. JORSTAD. Ann. Otol. Rhin. Laryng. *52:* 779, 1943.
NAVARRO-MARTIN, A. Actas Dermo-Sif. *32:* 271, 1940.
NOOJIN, R. O. and J. L. CALLAWAY. Arch. Dermatol. Syphilol. *47:* 620, 1943.
NOOJIN, R. O. and J. L. CALLAWAY. Arch. Dermatol. Syphilol. *49:* 305, 1944.
OGILVIE, W. H. Brit. Med. J. *2:* 254, 1940.
PFALZGRAF, C. München. Med. Wochenschr. *88:* 399, 1941.
REEVES, D. L., E. M. BUTT and R. W. HAMMACK. Arch. Int. Med. *68:* 57, 1941.
RUBBO, S. D. and J. M. GILLESPIE. Nature *146:* 838, 1940.
RUBBO, S. D. and J. M. GILLESPIE. Med. J. Australia *2:* 81, 1941.
SCHROEDER, C. B. Arch. Schiffs Tropen Hyg. *44:* 477, 1940.
SELBIE, F. R. Brit. J. Exptl. Path. *21:* 90, 1940.
SENTURIA, B. H. Laryngoscope *54:* 277, 1944.
SENTURIA, B. H. and F. T. WOLF. Arch. Otolaryngol. *41:* 56, 1945.
SIMON, E. Arch. Otolaryngol. *42:* 123, 1945.
TATUM, E. L. and G. W. BEADLE. Proc. Natl. Acad. Sci. *28:* 234, 1942.
TATUM, E. L. and A. C. GIESE. Arch. Biochem. *9:* 15, 1946.
VAN BREE, R. S. J. Mich. Med. Soc. *40:* 197, 1941.
WALKER, O. Lancet *1:* 1219, 1938.
WELCH, A. C. Physiol. Revs. *25:* 687, 1945.
WESSLER, S. and H. R. BROWNE. Ann. Internal Med. *22:* 886, 1945.
WILKINSON, E. E. J. Pediat. *18:* 805, 1941.
WOLF, F. T. Project Report 346, A.A.F.S.A.M., Randolph Field, Texas, 1945 (mimeographed). Also, Mycologia *38:* 213, 1946.
WOODS, D. D. Brit. J. Exptl. Path. *21:* 74, 1940.
WOODS, D. D. and P. FILDES. J. Soc. Chem. Ind. *59:* 133, 1940.

## II. ANTIBIOTIC AGENTS

**Introduction:**— The finding that certain metabolic products of microorganisms are inhibitory to the growth of pathogenic fungi is by no means a new one. The phenomenon of antagonism between fungi and other organisms has been known for many years, and the pathogenic fungi form no exception in this regard.

As examples of early work on microorganisms which have an antagonistic action upon the pathogenic fungi, we may consider the work of WEIDMAN (1927) and CHAMBERS and WEIDMAN (1928). WEIDMAN (1927) states, "Several years ago I noted that those portions of a certain ringworm fungus colony which were adjacent to a bacterial colony did not develop as luxuriantly as the more remote parts; the result was a lopsided fungus colony. A bacterium was present which restrained the growth of the fungus, but did not destroy it. . . . I tested out a number of yeast species obtained from normal feet, and finally secured one which inhibited the growth of *Epidermophyton cruris* when mixed with it and planted on culture mediums."

CHAMBERS and WEIDMAN (1928) isolated a hemolytic dull green organism identified as *Bacillus subtilis* from the skin of the normal toes of a large number of individuals. When inoculated upon suitable media together with the spores of pathogenic fungi, it was found that this organism completely inhibited, for a period of 2-3 months, the growth of several dermatophytes. *Sporotrichum schenckii* was similarly inhibited.

The antibiotic agents are substances formed as products of the metabolism of microorganisms, differing greatly from one another in origin, and in chemical and biological properties. Some, like penicillin, clavacin and gliotoxin, are produced by fungi; others, such as streptothricin and streptomycin, are produced by actinomycetes; still others, including tyrothricin and pyocyanine, are formed by bacteria. In the case of certain antibiotic agents, the conditions required for their production and even the chemical composition of the antibiotic principle are known, so that they can be exactly characterized as definite chemical compounds. In other instances, the preparations in use are merely concentrates of high activity, admixed in unknown amounts with extraneous substances, from which isolation of the active compound in a pure state has not yet been accomplished.

The necessity of providing a "yardstick" of the activity of such preparations has resulted in the establishment of "Oxford units," "streptothricin units," and the like, by which the activity of a particular antibiotic agent is defined in terms of its effect upon a particular test organism, such as *Staphylococcus aureus.* However necessary the system of unitage may be, it suffers a serious disadvantage in its inability to permit direct comparison of the activity of two different antibiotic agents upon any organism other than the one upon which the standards are based, a fact which must be constantly borne in mind by the reader. This is a consequence of the specific selective action of antibiotic agents.

According to REILLY, SCHATZ and WAKSMAN (1945) the effects of antibiotic substances upon fungi may include modifications in the morphology of the organism, changes in its physiology, pigment production or rate of growth, or complete destruction of the fungus.

Finally, the development of many of the antibiotic agents has been so recent that very little clinical information, dealing with the treatment of mycotic diseases, is yet available. The various substances will be discussed separately, on the basis of the information available in each case.

**Penicillin:**— The best known of the antibiotic agents, penicillin is produced by *Penicillium notatum, P. chrysogenum,* and certain other fungi. The discovery of penicillin by FLEMING, and the successes which have attended its use in bacterial infections, originally by FLOREY and later by many other workers, are so well known as not to require comment here.

Shortly after its antibacterial properties became generally known, experiments testing the action of penicillin against fungi were performed. ABRAHAM *et al.* (1941), using a preparation with an activity of 40-50 Oxford units per milligram, noted that complete inhibition of *Actinomyces bovis* occurred at a dilution of 1:1,000,000. Partial inhibition of this organism was obtained with penicillin 1:2,000,000, but none at 1:4,000,000. *A. bovis* is, therefore, very susceptible to penicillin. This observation was confirmed by FISHER (1943) in experiments using crude penicillin, before the purified material became available generally and in large quantities.

HOBBY, MEYER and CHAFFEE (1942) reported that *Cryptococcus hominis* was penicillin sensitive, but that the growth of *Candida (Monilia) albicans, C. krusei* and *C. candida* was not affected. The result of these workers with respect to *C. hominis* has not been verified by the subsequent results of others.

FOSTER and WOODRUFF (1943) found that *Candida albicans, C. stellatoidea, Trichophyton interdigitale, T. gypseum, T. schoenleinii, Epidermophyton floccosum, Microsporum felineum* and *Blastomyces dermatitidis* were unaffected by penicillin, even in dosages as high as 30 Oxford units per cc.

The most extensive work, however, concerning the action of penicillin upon pathogenic fungi is that of KEENEY, AJELLO and LANKFORD (1944). The organisms studied were *Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum, Microsporum audouini, M. felineum, Candida albicans, Blastomyces dermatitidis, Sporotrichum schenckii, Cryptococcus neoformans, Coccidioides immitis, Phialophora pedrosoi* and *Histoplasma capsulatum.* The organisms were grown in dextrose broth containing 0.1, 0.5, 1, and 10 Oxford units of penicillin per cc. Because penicillin is unstable under some conditions, fresh penicillin was added every 48 hours, so that the original concentrations were maintained for the duration of the 14 day period of observation. No inhibition of growth of any of these fungi was noted.

KEENEY *et al.* (1944) also studied the action of penicillin upon *Actinomyces bovis,* grown in thioglycollate broth containing 0.005-10 Oxford units per cc. This organism was inhibited and apparently killed by a concentration of 0.01 Oxford unit per cc., thus confirming the results of previous workers as to the susceptibility of *A. bovis* to penicillin.

The clinical use of penicillin in cases of actinomycosis, especially when employed as an adjunct to surgical intervention, has met with some success (KEENEY *et al.,* 1944; SHULMAN, 1944) but it is perhaps too early as yet to form a definite opinion as to its value. The failure of attempted penicillin therapy in the treatment of coccidioidomycosis has been reported by MICHAEL, MCLAUGHLIN and CENAC (1944).

**Streptothricin and Streptomycin:**— It is perhaps desirable to consider together the results obtained with streptothricin and streptomycin, two of the antibiotic agents derived from actinomycetes. Streptothricin, produced by *Actinomyces lavendulae,* and streptomycin, from *A. griseus,* were isolated and studied by WAKSMAN and his co-workers.

The action of streptothricin upon a considerable number of yeasts, saprophytic fungi and pathogenic fungi was studied by FOSTER and WOODRUFF (1943). The "streptothricin unit" employs *Bacillus subtilis* as the test organism. The results of FOSTER and WOODRUFF (1943) with pathogenic fungi are presented in the following table:

TABLE 5: *Action of streptothricin upon pathogenic fungi* (*after* FOSTER and WOODRUFF, 1943) :—

| ORGANISM | *Streptothricin units per cc.* | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | .1 | 1 | 10 | 30 | 100 | 300 |
| *Blastomyces dermatitidis* | + | + | + | 0 | 0 | 0 | 0 |
| *Trichophyton schoenleinii* | + | + | + | + | — | 0 | 0 |
| *Epidermophyton floccosum* | + | + | + | + | + | — | — |
| *Trichophyton interdigitale* | + | + | + | + | + | + | 0 |
| *Candida albicans* | + | + | + | + | + | + | + |
| *Microsporum felineum* | + | + | + | + | + | + | + |
| *Trichophyton gypseum* | + | + | + | + | + | + | + |

Thus, streptothricin has considerable activity against pathogenic fungi. *Blastomyces dermatitidis* and *Trichophyton schoenleinii* were inhibited by streptothricin in concentrations comparing favorably with that required for the inhibition of *Escherichia coli.* The remaining pathogenic fungi tested were relatively insensitive to streptothricin, some being unaffected by 100 times the concentration required to inhibit *E. coli.*

Streptomycin, on the other hand, is almost completely inactive against fungi. ROBINSON, SMITH and GRAESSLE (1944) compared the action of streptothricin and streptomycin upon a number of pathogenic fungi. The "streptothricin unit" was defined as the quantity, in 1 cc. of nutrient broth or agar, which will just inhibit growth of *E. coli.* The streptomycin preparation used by ROBINSON *et al.* (1944) had a potency of 27-100 units per milligram. Their results are presented in the following table:

TABLE 6: *Comparison of the action of streptomycin and streptothricin upon pathogenic fungi* (ROBINSON, SMITH and GRAESSLE, 1944) :—

| ORGANISM | *Units per cc. required to cause inhibition* | |
|---|---|---|
| | *Streptomycin* | *Streptothricin* |
| *Cryptococcus neoformans* | > 4,000 | 250 |
| *Sporotrichum schenckii* | > 4,000 | 250 |
| *Trichophyton gypseum* | 3,500 | 500 |
| *Trichophyton interdigitale* | 4,000 | 1,000 |
| *Microsporum canis* | 4,000 | 1,000 |
| *Epidermophyton floccosum* | > 4,000 | 1,000 |

Although streptomycin has little effect upon pathogenic fungi generally, it would appear from the work of SCHATZ and WAKSMAN (1944) that certain actinomycetes are highly sensitive to this compound. *Actinomyces* (*Streptomyces*) *albus,* isolated from a case of otomycosis, was completely inhibited for three days by 0.4—1.25 units of streptomycin per cc. *Actinomyces* (*Nocardia*) *asteroides* and *A.* (*N.*)*gypsoides,* however, were much more resistant to streptomycin. No data are yet at hand concerning the action of streptomycin on *Actinomyces bovis.*

REILLY, SCHATZ and WAKSMAN (1945) have compared the activity of several antibiotic agents upon *Candida albicans, Trichophyton mentagrophytes,* and *Cryptococcus neoformans.* Streptomycin was less toxic to these fungi than gliotoxin, actinomycin, clavacin, fumigacin, streptothricin, or chaetomin. Streptothricin, however, is far more active against fungi, and shows promise clinically.

**Gliotoxin:**— The antagonism between *Trichoderma* and soil microorganisms has long been known to microbiologists. The potent antibiotic agent produced by *Gliocladium fimbriatum,* strains of *Trichoderma* and certain other fungi was isolated by WEINDLING (1941), and is now known as gliotoxin. WEINDLING reported that the fungicidal activity of this substance to *Rhizoctonia solani* was intermediate between that of copper sulphate and that of mercuric chloride. Thus from the very beginning gliotoxin was known as an antifungal compound, differing in this respect from other antibiotic agents effective against bacteria, whose antifungal properties were regarded as incidental or of definitely secondary importance.

Methods for the large scale production of gliotoxin, information as to its physical and chemical properties, and the first data as to its action upon pathogenic fungi were provided by JOHNSON, BRUCE and DUTCHER (1943). Tests showed that 15.6 mg. of gliotoxin per cc. inhibited the growth of *Epidermophyton* in broth culture, and 3.9 mg. per cc. inhibited the growth of this organism in an agar medium. *Blastomyces dermatitidis* failed to grow in a medium containing gliotoxin 1:8,000.

HERRICK (1945) has determined the fungistatic and fungicidal effects of gliotoxin upon *Trichophyton gypseum.* Growth of this organism on Sabouraud's agar was completely inhibited by 0.001% gliotoxin, but no inhibition occurred at 0.0001%. Spores suspended in gliotoxin solutions, removed by centrifugation, washed and transferred to agar media were unharmed after 24 hours exposure to 0.001% gliotoxin. Two hours exposure to a 0.01% solution, however, was lethal to more than 99% of the spores of *T. gypseum.*

REILLY, SCHATZ and WAKSMAN (1945) have studied the fungistatic activity of gliotoxin, in comparison with other antibiotic agents from fungi and actinomycetes, upon *Candida albicans, Cryptococcus neoformans,* and two strains of *Trichophyton mentagrophytes.* The figures presented in the following table indicate fungistatic activity on a unit per cc. basis for one gram of the material. The units for each antibiotic agent are of course different:

TABLE 7: *The activity of various antibiotic agents upon C. albicans, C. neoformans and T. mentagrophytes (after* REILLY, SCHATZ and WAKSMAN, 1945) :—

| ANTIBIOTIC | *C. albicans* | *T. mentagrophytes* | | *C. neoformans* |
|---|---|---|---|---|
| | | 598 | 640 | |
| Gliotoxin | 4,000,000 | 6,000,000 | 2,000,000 | > 20,000,000 |
| Actinomycin | < 500,000 | 5,000,000 | 1,500,000 | 1,500,000 |
| Clavacin | 3,300 | 33,000 | 25,000 | 10,000 |
| Fumigacin | 8,900 | 14,800 | 8,900 | 30,000 |
| Streptothricin | < 60 | 4,500 | 4,500 | 12,000 |
| Chaetomin | < 4,000 | < 4,000 | < 4,000 | < 4,000 |
| Streptomycin | < 45 | < 45 | < 45 | < 45 |

Insofar as its action upon fungi is concerned, gliotoxin is thus the most potent antibiotic substance yet discovered. Notwithstanding its high toxicity to animals, it may have promise clinically, when applied locally to mycotic lesions.

**Clavacin**:— Clavacin is an antibiotic agent derived from *Aspergillus clavatus* by WAKSMAN, HORNING and SPENCER (1943). It has been isolated in a pure state, and its structure determined as that of an unsaturated ketone. The empirical formula of clavacin is $C_7H_6O_4$.

WAKSMAN, HORNING and SPENCER (1943) noted that clavacin has rather limited fungistatic properties. The fungi with which clavacin was tested by them are not named, but it is noted that they "appeared to overcome the effect in a short time, either by inactivating the clavacin or by adjusting themselves to its action." KATZMAN *et al.* (1944) reported that clavacin had considerable activity against *Rhizopus nigricans, Saccharomyces cerevisiae, Candida albicans* and *Sporotrichum schenckii.*

The fungistatic and fungicidal properties of clavacin upon *Candida albicans, Oidium asteroides* and *Trichophyton gypseum* have been recently studied by HERRICK (1945). The clavacin was produced from *A. clavatus* according to the method of WAKSMAN, HORNING and SPENCER; in addition, the product of commercial drug houses was used. *T. gypseum* proved to be more susceptible to clavacin than either *Candida* or *Oidium,* a 0.001% solution causing slight inhibition of growth, and 0.02 or 0.04% concentration checking growth of *T. gypseum* completely. These concentrations had only a slight inhibitory effect on *C. albicans* and *O. asteroides.*

0.1% clavacin did not kill *C. albicans* or *O. asteroides* even after

24 hours, but was lethal to *T. gypseum* in 2-5 hours. 0.5 and 1% solutions were fungicidal for all organisms, 1% clavacin killing spores of *T. gypseum* within 15 minutes, *C. albicans* in 30 minutes, and *O. asteroides* in one hour.

REILLY, SCHATZ and WAKSMAN (1945) noted that clavacin was an effective fungistatic agent against *Candida albicans, Cryptococcus neoformans* and *Trichophyton mentagrophytes,* inferior only to gliotoxin and actinomycin, but they see little promise for the clinical use of this compound because of its high toxicity to animals.

GEIGER and CONN (1945) determined that the antibiotic activity of clavacin was due to the unsaturated group in the molecule. Synthetic unsaturated ketones such as acrylophenone were found to resemble clavacin in fungistatic properties. These workers presented evidence supporting the hypothesis that the antibiotic activity of clavacin is due to its reaction with sulfhydryl groups of either enzyme systems of the microorganisms or some of their essential metabolites.

**Pyocyanine and Hemipyocyanine:**—*Bacillus pyocyaneus,* more properly known as *Pseudomonas aeruginosa,* is an organism from which a number of substances having antibiotic activity have been isolated. Among these are pyocyanine and hemipyocyanine (alphahydroxy phenazine), which have been known for many years.

STOKES, PECK and WOODWARD (1942) have studied the growth-inhibitory action of pyocyanine and hemipyocyanine upon yeasts and pathogenic fungi. The hemipyocyanine used by them was prepared by synthetic methods, while the pyocyanine was isolated as the perchlorate from cultures of *Ps. aeruginosa.* The results of this study upon *Candida albicans, Trichophyton (Achorion) schoenleinii, Microsporum gypseum* and *Trichophyton gypseum,* grown on Sabouraud's agar, are presented in Table 8.

Thus, pyocyanine has relatively little action upon the growth of pathogenic fungi. Hemipyocyanine, however, in dilutions as high as 1:20,000 completely inhibited the growth of all the fungi tested, and *T. schoenleinii* was inhibited by hemipyocyanine 1:60,000. Essentially similar results were obtained with a number of yeast species.

Hemipyocyanine has a fungistatic potency superior to most of the commonly used disinfectants, including mercuric chloride, phenol, cresol, salicylic acid and mercurochrome, but inferior to crystal or gentian violet. It cannot be used intravenously, according to STOKES, PECK and WOODWARD (1942) but possibly could be employed in topical applications in the treatment of superficial fungus infections.

**Tyrothricin:**— Tyrothricin, an antibiotic substance isolated from the spore-forming soil organism *Bacillus brevis,* was discovered by DUBOS in 1939. Chemical studies soon disclosed that tyrothricin was not a single substance, but a mixture of two materials known as

TABLE 8: *Action of pyocyanine and hemipyocyanine upon pathogenic fungi* (STOKES, PECK and WOODWARD, 1942) :—

| DILUTION OF ANTIBIOTIC | *Trichophyton schoenlcinii* | *Micro-sporum gypseum* | *Trichophyton gypseum* | *Candida albicans* |
|---|---|---|---|---|
| | *Pyocyanine* | | | |
| 1 :2,000 | — | — | — | ++ |
| 1 :5,000 | — | + | + | +++ |
| 1 :10,000 | ++ | ++ | ++ | +++ |
| 1 :20,000 | ++ | ++ | ++ | ++++ |
| 1 :40,000 | ++ | +++ | ++++ | ++++ |
| 1 :60,000 | ++++ | +++ | ++++ | ++++ |
| 1 :80,000 | ++++ | ++++ | ++++ | ++++ |
| 1 :100,000 | ++++ | ++++ | ++++ | ++++ |
| Control | ++++ | ++++ | ++++ | ++++ |
| | *Hemipyocyanine* | | | |
| 1 :2,000 | — | — | — | — |
| 1 :5,000 | — | — | — | — |
| 1 :10,000 | — | — | — | — |
| 1 :20,000 | — | — | — | — |
| 1 :40,000 | — | — | — | ++ |
| 1 :60,000 | — | ++ | + | ++++ |
| 1 :80,000 | ++ | ++++ | + | ++++ |
| 1 :100,000 | +++ | ++++ | ++ | ++++ |
| Control | ++++ | ++++ | ++++ | ++++ |

tyrocidine and gramicidin, both of which are polypeptides. It has appeared undesirable to separate these two fractions for clinical use because of their complementary spectra of activity. Tyrothricin produces hemolysis and hence cannot be employed parenterally.

STOKES, PECK and WOODWARD (1942) have studied the action of tyrothricin upon the growth of *Trichophyton* (*Achorion*) *schoenleinii, Microsporum gypseum, Trichophyton gypseum* and *Candida albicans* on Sabouraud's agar. The results are presented in Table 9.

TABLE 9: *Action of tyrothricin upon pathogenic fungi* (STOKES, PECK and WOODWARD, 1942) :—

| DILUTION OF TYROTHRICIN | *Trichophyton schoenleinii* | *Micro-sporum gypseum* | *Trichophyton gypseum* | *Candida albicans* |
|---|---|---|---|---|
| 1 :2,000 | — | — | — | — |
| 1 :5,000 | — | — | — | — |
| 1 :10,000 | — | — | — | ++++ |
| 1 :20,000 | — | + | + | ++++ |
| 1 :40,000 | + | ++ | + | ++++ |
| 1 :60,000 | + | ++ | + | ++++ |
| 1 :80,000 | ++++ | +++ | +++ | ++++ |
| 1 :100,000 | ++++ | +++ | +++ | ++++ |
| Control | ++++ | ++++ | ++++ | ++++ |

Tyrothricin in dilutions of 1:5,000-1:20,000 completely inhibited the growth of all of the pathogenic fungi tested. The fungistatic activity of tyrothricin is thus approximately equal to that of commonly used disinfectants, and STOKES, PECK and WOODWARD (1942) suggest that it may be of value in the treatment of superficial fungus infections.

**Miscellaneous Antibiotics:**— Actinomycin is an antibiotic agent produced by *Actinomyces antibioticus.* Its activity against *Candida albicans, Cryptococcus neoformans* and *Trichophyton mentagrophytes* was found by REILLY, SCHATZ and WAKSMAN (1945) to be very high, inferior only to gliotoxin. Because actinomycin is highly toxic to animals, however, it probably cannot be used clinically.

Fumigacin, from *Aspergillus fumigatus,* was found by WAKSMAN, HORNING and SPENCER (1943) to possess a limited activity against fungi. REILLY, SCHATZ and WAKSMAN (1945) found that the activity of fumigacin against *Candida albicans, Cryptococcus neoformans,* and *Trichophyton mentagrophytes* was generally less than that of gliotoxin, actinomycin or clavacin, and do not regard fumigacin as having activity sufficient for clinical use.

Chaetomin, from *Chaetomium cochlioides,* was found by REILLY *et al.* (1945) to be almost completely inactive against the pathogens *Candida albicans, Cryptococcus neoformans* and *Trichophyton mentagrophytes.*

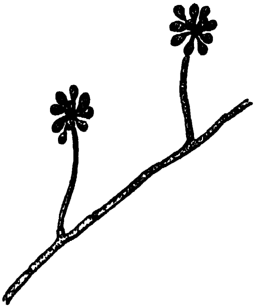
The activity of penicillic acid, an antibiotic substance produced by *Penicillium puberulum* and *P. cyclopium,* upon a number of saprophytic fungi has been determined by GEIGER and CONN (1945) as being much less than that of clavacin.

Subtilin, an antibiotic agent from *Bacillus subtilis,* was found by SALLE and JANN (1945) to inhibit the growth of *Actinomyces asteroides, A. pelletieri,* and *Nocardia mexicana* when present in Sabouraud's agar in the proportion 1:1000. *Candida albicans, Cryptococcus neoformans, Sporotrichum schenckii* and *Trichophyton gypseum,* on the other hand, were not affected by this quantity of subtilin.

Thus, during the very brief period in which attention has been devoted to the action of antibiotic agents upon the pathogenic fungi, substances have been found which are effective *in vitro* against a number of pathogens. *Actinomyces bovis* is inhibited by penicillin, *Candida albicans* by gliotoxin, clavacin, actinomycin and hemipyocyanine, *Cryptococcus neoformans* by gliotoxin, clavacin and actinomycin, *Blastomyces dermatitidis* by gliotoxin and streptothricin, *Sporotrichum schenckii* by clavacin, *Trichophyton (Achorion) schoenleinii* by streptothricin, tyrothricin and hemipyocyanine, *Trichophyton mentagrophytes (gypseum)* by gliotoxin, clavacin, actinomycin, tyrothricin and hemipyocyanine, and *Microsporum gypseum* by tyrothricin and hemipyocyanine. The development of new antibiotic agents, and the possibilities of practical use of findings such as these must be left to the future.

*Literature Cited:* —

ABRAHAM, E. P., E. CHAIN, C. M. FLETCHER, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS and H. W. FLOREY. Lancet *2:* 177, 1941.
CHAMBERS, S. O. and F. D. WEIDMAN. Arch. Derm. Syph. *18:* 568, 1928.
FISHER, A. M. Bull. Johns Hopkins Hosp. *73:* 343, 1943.
FOSTER, J. W. and H. B. WOODRUFF. Arch. Biochem. *3:* 241, 1943.
GEIGER, W. B. and J. E. CONN. J. Amer. Chem. Soc. *67:* 112, 1945.
HERRICK, J. A. Proc. Soc. Exptl. Biol. Med. *59:* 41, 1945.
HERRICK, J. A. Ohio J. Sci. *45:* 45, 1945.
HOBBY, G. L., K. MEYER and E. CHAFFEE. Proc. Soc. Exptl. Biol. Med. *50:* 277, 1942.
JOHNSON, J. R., W. F. BRUCE and J. D. DUTCHER. J. Amer. Chem. Soc. *65:* 2005, 1943.
KATZMAN, P. A., E. E. HAYS, C. K. CAIN, J. J. VAN WYK, F. J. REITHEL, S. A. THAYER, E. A. DOISY, W. S. GABY, C. J. CARROLL, R. D. MUIR, L. R. JONES and N. J. WADE. J. Biol. Chem. *154:* 475, 1944.
KEENEY, E. L., L. AJELLO and E. LANKFORD. Bull. Johns Hopkins Hosp. *75:* 410, 1944.
MICHAEL, P., R. F. MCLAUGHLIN and P. L. CENAC. U. S. Nav. Med. Bull. *43:* 122, 1944.
REILLY, H. C., A. SCHATZ and S. A. WAKSMAN. J. Bact. *49:* 585, 1945.
ROBINSON, H. J., D. G. SMITH and O. E. GRAESSLE. Proc. Soc. Exptl. Biol. Med. *57:* 226, 1944.
SALLE, A. J. and G. J. JANN. Proc. Soc. Exptl. Biol. Med *60:* 60, 1945.
SCHATZ, A. and S. A. WAKSMAN. Proc. Soc. Exptl. Biol. Med. *57:* 244, 1944.
SHULMAN, H. M. Bull. Vet. Admin. *21:* 230, 1944.
STOKES, J. L., R. L. PECK and C. R. WOODWARD, Jr. Proc. Soc. Exptl. Biol. Med. *51:* 126, 1942.
WAKSMAN, S. A., E. S. HORNING and E. L. SPENCER. J. Bact. *45:* 233, 1943.
WEIDMAN, F. D. Arch. Derm. Syph. *15:* 415, 1927.
WEINDLING, R. Phytopath. *31:* 991, 1941.



## *Chapter 8*

# GEOGRAPHIC DISTRIBUTION OF SYSTEMIC FUNGUS DISEASES

*by*

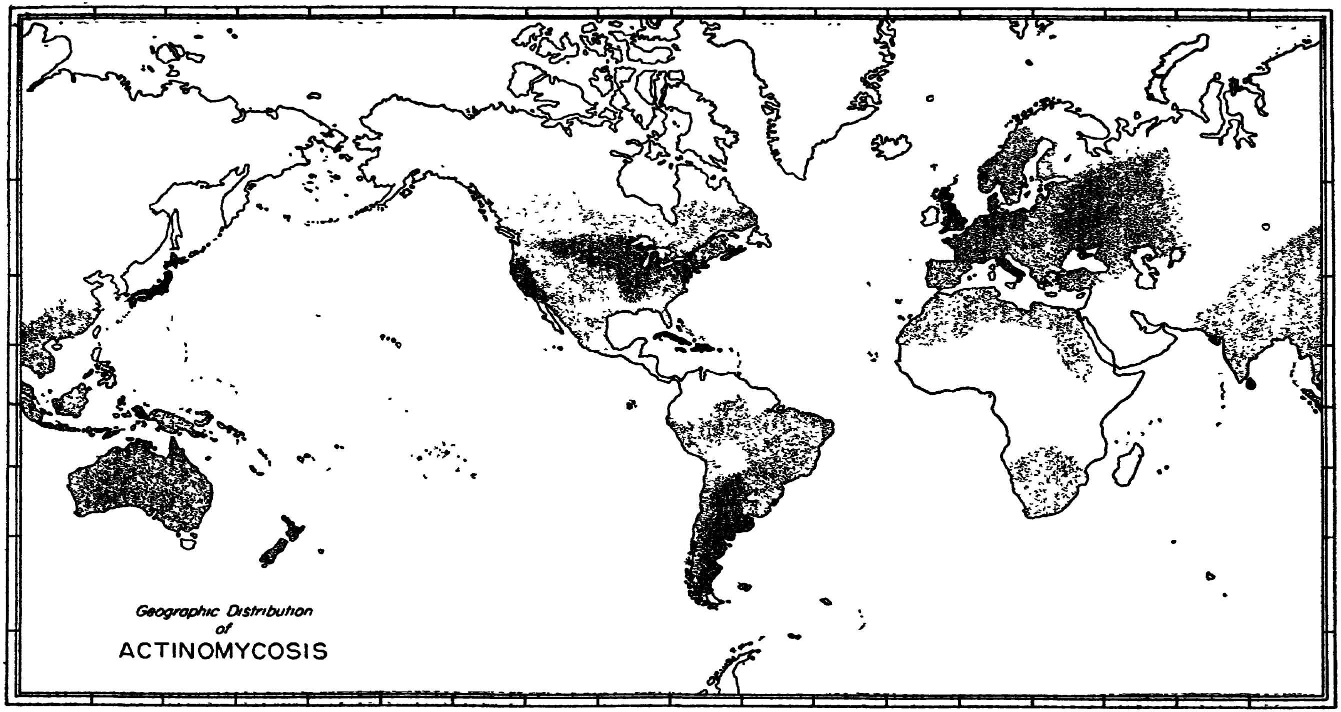
DONALD S. MARTIN

Data on the geographic distribution of the systemic mycoses are very meager when compared with the extensive knowledge that has accumulated concerning the distribution of diseases caused by other types of infectious agents. Systemic fungus infections are transmitted from man to man only under exceptional circumstances, and the lack of contagiousness is one of the more important reasons why so few epidemiologic studies of fungus diseases have been made. However, during recent years it has been recognized that some of the mycoses are sufficiently widespread to present real and important public health problems. Intensive studies of coccidioidomycosis have shown that infection with *Coccidioides immitis* has a much wider geographic distribution than was thought formerly and that the incidence of the infection in endemic areas is extremely high (SMITH, 1940). Evidence available at the present time points to the possibility that histoplasmosis may be relatively common in a benign form in the Mississippi Valley area of the United States (PALMER, 1945).

Except for actinomycosis few, if any, of the fungus diseases are reportable to various health departments, and sources of information such as the master tables of causes of death published by the Bureau of the Census are of no aid because none of the table headings contains any reference either to a specific fungus disease or to mycoses as a group.

Although published case reports are of value in attempting to determine areas in which fungus infections may be found, they are of little value in estimating the prevalence of any one disease in such areas. Undoubtedly, many cases are not reported because the disease either is unrecognized or, if recognized, the physician or laboratory shrinks from the task of untangling the numerous systems of terminology and methods of classification that have been advocated by various workers in the field. For this reason, there is a tendency for reported cases to be clustered geographically around certain areas in which there are clinics or laboratories with special interests in the problem of fungus infection.

The lack of standard methods of identification and classification introduces additional confusing elements into the problem of mapping



— FIGURE 1 —

the distribution of specific fungous diseases. In some of the more important mycoses, the etiologic agents are described under a variety of different names, depending upon the opinion of the author or collaborating mycologist. On the other hand, certain infections caused by specifically different and unrelated fungi are grouped together under a common clinical term. For example, the name blastomycosis is used commonly to designate infections caused by *Blastomyces dermatitidis, Cryptococcus neoformans* and *Candida* (*Monilia*) *albicans* in spite of the fact that the lesions in each infection are quite different and the fungi are separated easily by cultural methods. The use of the term "European blastomycosis" to designate infections caused by *Cryptococcus* serves as a method of identifying the infection, but the term is misleading in that it implies that the disease has a predominantly European distribution.

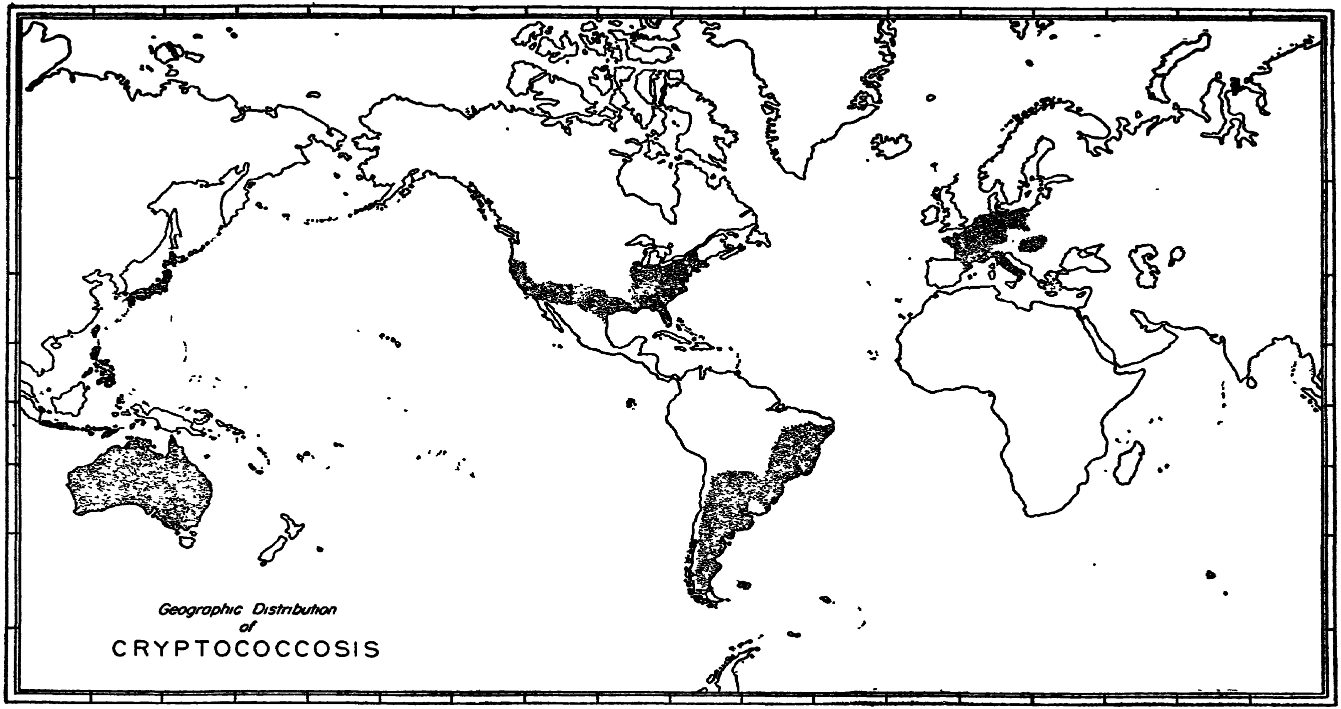
With the above qualifications, an attempt has been made to estimate the areas in which specific fungus diseases have been reported. For convenience in discussion, the systemic mycoses are discussed under three general headings: (*1*) infections, presumably endogenous in origin, in which the distribution is world-wide; (*2*) infections of exogenous origin in which the geographic distribution is world-wide but spotty; and (*3*) infections of exogenous origin which are characterized by being reported only from rather sharply limited geographic areas.

**Endogenous Infections:**—Included in this group are three fungus diseases in which the evidence indicates that the infection is acquired from some focus in the body rather than from an extraneous source in nature. Such infections would be expected to have a world-wide distribution.

*Actinomycosis.*—Actinomycosis is the most ubiquitous of all systemic mycoses, and cases have been reported from every part of the world (*see* Fig. 1).

The anaerobic fungus *A. bovis* has been shown to be a common inhabitant around the teeth, gums, and tonsils in a high percentage of individuals who show no evidence of serious infection (SLACK, 1942). The organism, thus, is in a position to initiate severe infection under a variety of circumstances, such as invasion of the mucosa to produce cervicofacial actinomycosis or through aspiration or swallowing, to result in pulmonary or abdominal infections.

Of all the systemic mycoses, actinomycosis is probably the easiest infection to diagnose clinically because it often presents very characteristic clinical features which are well known to most physicians. Furthermore, the diagnosis is established readily by hematoxylin and eosin-stained sections obtained at biopsy or necropsy. In such sec-



— FIGURE 2 —

tions the sulfur granules are large and brilliantly stained and are not apt to be confused with any other infection.

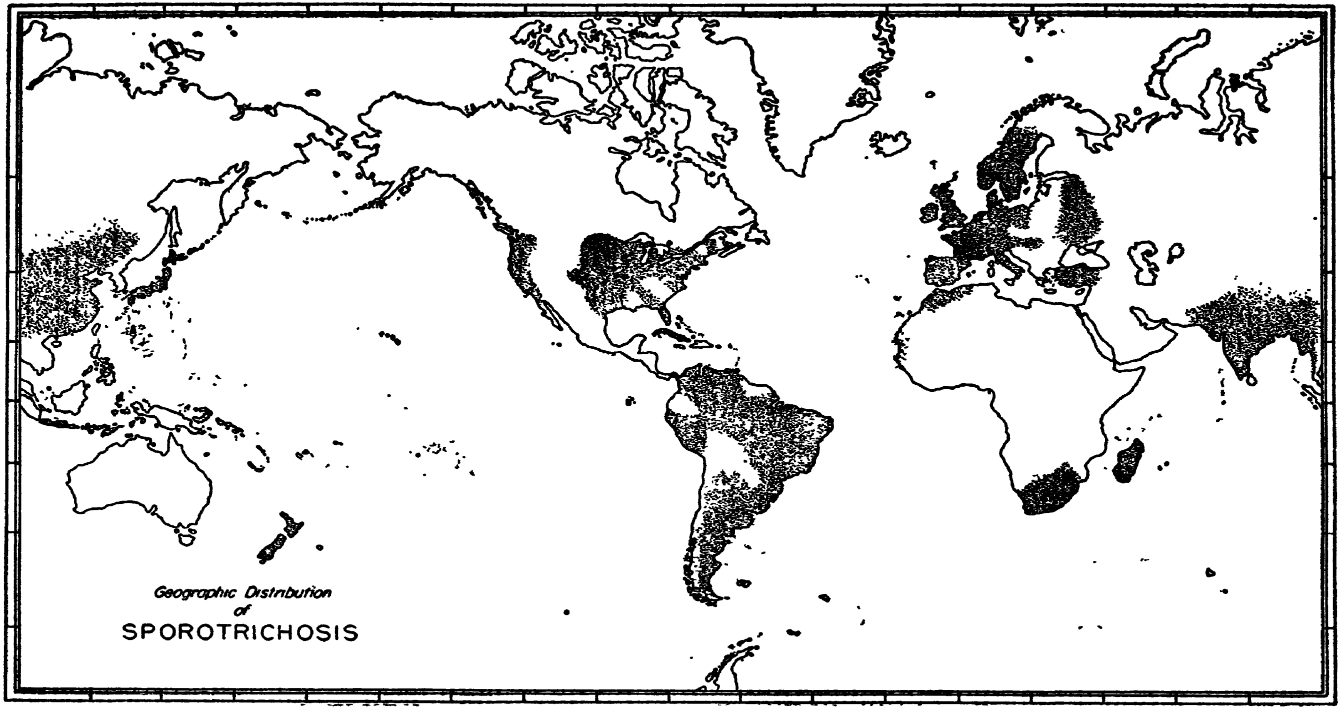
It is felt that both of the factors mentioned above play a contributory rôle in making actinomycosis a disease which is known and reported from all parts of the world.

*Moniliasis.* — Infection with the yeast-like fungus *Candida* (*Monilia*) *albicans* occurs also in all parts of the world, but the organism produces such a variety of clinical syndromes that it is impractical to attempt an allocation of any type of infection to any particular geographic area. Bronchopulmonary moniliasis, first thought to be a tropical disease, now is known to occur in many other climates (CONANT, MARTIN, SMITH, BAKER, and CALLAWAY, 1944). Predisposition to infection is dependent more upon personal factors than climatic conditions. Like *Actinomyces,* the fungus is harbored by apparently normal individuals; it has been isolated from the skin, oral cavities, and feces. The fungus can produce thrush, vaginitis, and various types of infection in the skin, nails, lungs, and meninges, and is often a secondary invader in other conditions. The organism may be present in the sputa of tuberculous patients, in the feces of individuals with sprue, and in the lesions of perlèche.

Although *C. albicans* is the primary etiologic agent in many clinical syndromes, it often is difficult to evaluate the specific rôle of the fungus and one is left with the impression that *Candida* (*Monilia*) is capable of causing almost any type of infection in almost any part of the world.

*Cryptococcosis.* — It is not known whether or not diseases caused by *Torulopsis* (*Cryptococcus*) *neoformans* should be included among infections of endogenous origin. Strains of the fungus, pathogenic for animals, have been isolated from certain fermenting fruit juices, but there is no direct evidence that man has contracted an infection from such a source (SANFELICE, 1895). Species of *Torulopsis* have been isolated from normal skin and feces. Insufficient work has been done on these strains isolated from normal individuals to establish their relationship to the disease. Some of the species of *Torulopsis* are pigmented; others are known to be non-pathogenic, but it is not unlikely that a few of them may acquire pathogenicity under certain conditions and initiate infection.

Cases have been reported from the United States, mostly from the eastern and southern parts, from Germany, Italy, France, Brazil, Argentina, and Paraguay. In the southwest Pacific region, cases have been reported from Japan, the Philippines, Dutch East Indies, and Australia. There is considerable clinical evidence that the portal of entry may be the lungs, and we have seen cases with pulmonary lesions by x-ray in which symptoms were minimal. It is possible that many



— FIGURE 3 —

pulmonary infections exist unrecognized because of the lack of symptoms. Unfortunately, there are no simple methods of testing for subclinical infections analogous to those which have proven so successful in studying the epidemiology of coccidioidomycosis.

The spotty distribution in various parts of the world (Fig. 2) can be correlated with a known interest in the disease and does not necessarily indicate that the infection is limited to these areas.

**Exogenous Infections with Widespread Geographic Distribution:** — Although the exact sources in nature of the infections included in this group have not been established, certain features of case histories and the location of lesions suggest an exogenous origin.

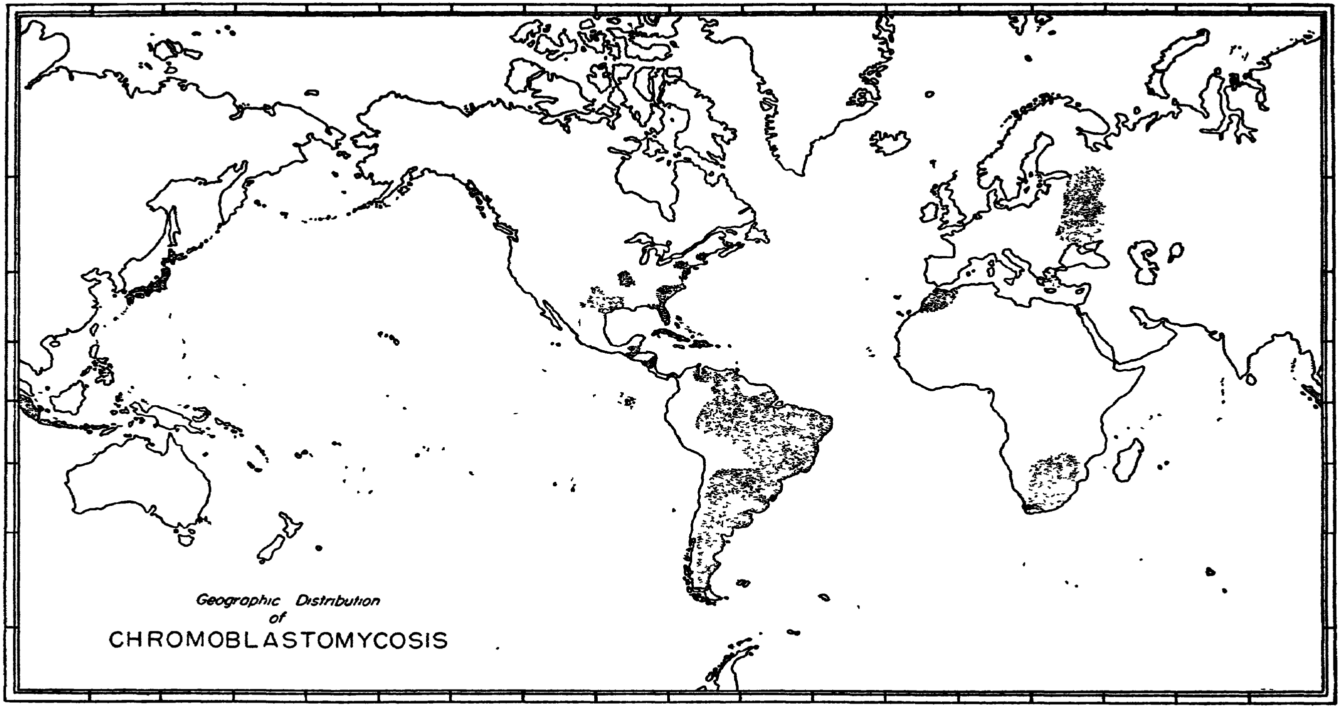
*Sporotrichosis.* — This infection has been reported from practically all parts of the world, but the recorded cases do not indicate a coverage as complete as that of actinomycosis. Large numbers of cases have been reported from some areas, reflecting again the interest of certain clinics in the particular mycosis. Thus, many cases have been reported from France, South Africa, and the upper Mississippi Valley in the United States (*see* Fig. 3). As an illustration of the intensity of infection in certain areas or under peculiar conditions, it is of interest to cite a recent publication where 650 cases were reported in men working in a mine shaft in the Transvaal area in South Africa (DU TOIT, 1942).

Like actinomycosis, sporotrichosis has certain characteristics by which a clinical diagnosis can be made quite easily. The response to treatment is so satisfactory that it is probable that only rare or unusual cases now are considered worth reporting.

*Chromoblastomycosis.* — Like sporotrichosis, chromoblastomycosis has been reported from widely scattered parts of the world, but unlike infections due to *Sporotrichum,* the number of cases reported from any one area is small. Instances of chromoblastomycosis have been found in various parts of the United States, Cuba, Puerto Rico, Santo Domingo, Guatemala, Costa Rica, the Canal Zone, Venezuela, Brazil, Argentina, Paraguay, and Uruguay. The only case reported from Europe was found in Russia (TSCHERNJAWSKI, 1929). In Africa, there have been instances of the disease reported in Algeria and southern Africa. In the Pacific area, cases have been recorded in Java, Sumatra, and Japan (*see* Fig. 4).

That the infection is exogenous in origin has been suspected from clinical histories and the location of the primary lesion which is usually on an extremity. Furthermore, a fungus morphologically indistinguishable and antigenically related to *Phialophora verrucosa* has been isolated from wood pulp (MARTIN, 1938).

Several different fungi can cause the clinical picture of chromoblastomycosis, and at one time it was thought that the etiologic agent



— FIGURE 4 —

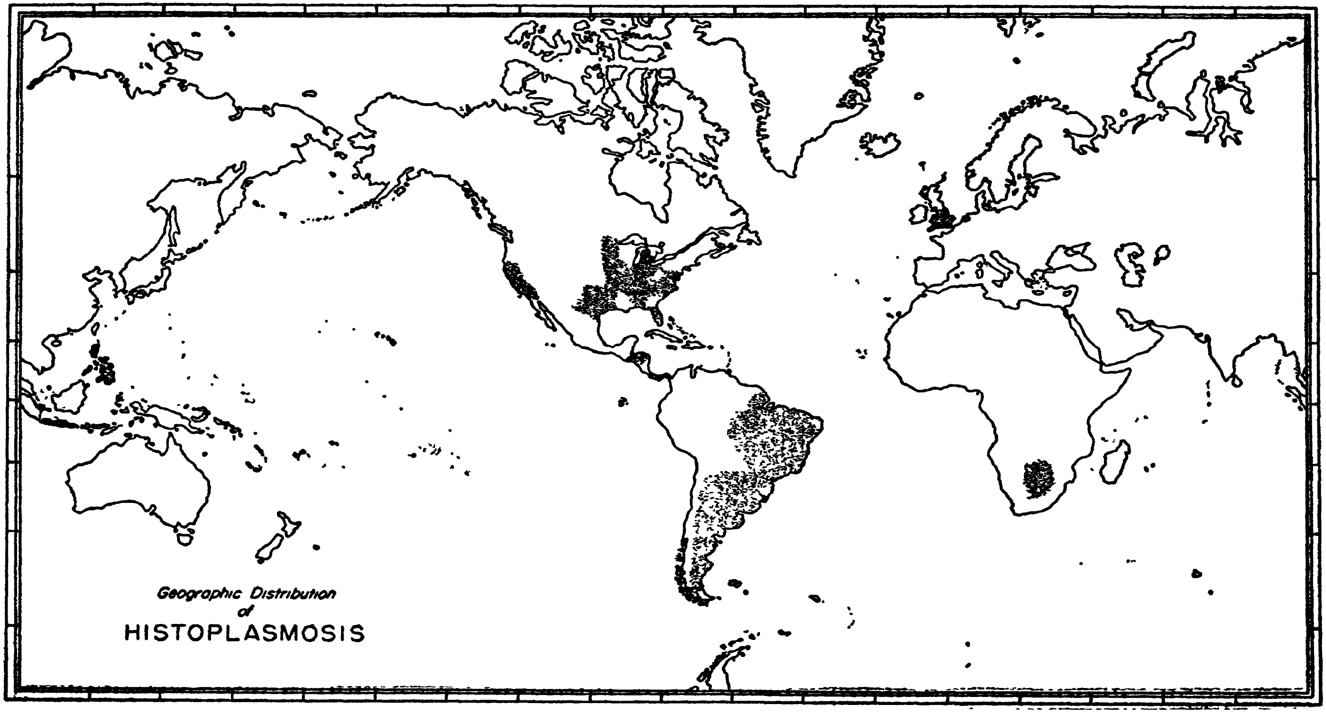
of the disease in North America was *Phialophora verrucosa* and that the South American form of the infection was caused by *Hormodendrum Pedrosoi.* As more cases have been studied, it has been learned that there is no relationship between the fungus and the area in which the infection is acquired. A third fungus, *Hormodendrum compactum,* has been isolated from only one patient whose infection was acquired in Puerto Rico (CARRIÓN, 1936).

*Histoplasmosis.* — Like chromoblastomycosis, the reports of infection with *Histoplasma capsulatum* have come from many parts of the world, such as the United States, Central America, South America, England, South Africa, the Philippines, and Java (*see* Fig. 5). In North America, by far the greater number of cases have been found in the North Central States with a scattering of cases in the East and South. All of the above data have been based on case reports of severe infections, most of which terminated fatally.

A recent survey made of student nurses in the North Central States revealed a high percentage of individuals with calcified lesions in the lungs and hilar lymph nodes who showed a negative tuberculin but a positive skin test to an extract of *Histoplasma capsulatum* (PALMER, 1945). Further work will have to be done before concluding that such positive skin tests represent actual proof of past infection with the fungus. However, if such proof can be obtained, additional surveys should show the true geographic distribution of infection and may reveal that primary invasion by the fungus is much more common than now realized.

**Exogenous Infections with Limited Geographic Distribution:** — *Coccidioidomycosis.* — Except for three cases reported from Italy and South America, coccidioidomycosis is limited essentially to the southwestern part of the United States. The infection definitely is acquired from an exogenous source, and the fungus has been isolated from the soil in the endemic area. It has been shown recently that certain rodents are naturally infected with the fungus and may represent a reservoir in nature from which the soil is contaminated (EMMONS, 1943).

The discovery that *Coccidioides immitis* causes such an extremely high incidence of infection in the population of endemic areas was a startling observation, and was the first demonstration of a mycosis which was sufficiently important to present a public health problem (SMITH, 1940). Skin tests with coccidioidin have shown that the endemic areas extend as far east as western Texas (MARCUSE and KAROTKIN, 1944). Although surveys have not been conducted in all parts of the United States, tests in sample areas in the northern and eastern parts of the country have shown positive reactions very



— FIGURE 5 —

rarely. As far as is known, surveys of this nature have not been made in other parts of the world.

*North American blastomycosis.* — This infection is limited essentially to the United States. Except for several cases in Canada and one in England, there have been no authenticated cases reported outside the continental United States. The authors recording the English case commented on the fact that their patient was engaged in the occupation of opening packing boxes which had been shipped to England from the United States (Dowling and Elsworthy, 1925).

In the United States, there is a rather peculiar distribution, most cases having been reported from the Central and South Atlantic States. Few cases have been reported from the New England and Middle Atlantic States. There have been several reported in the Pacific States, but we are unaware of any originating in the Mountain States (*see* Fig. 6).

The high incidence of the infection in males and the clinical histories suggest that the infection is acquired from some source in nature, but proof of this assumption is lacking.

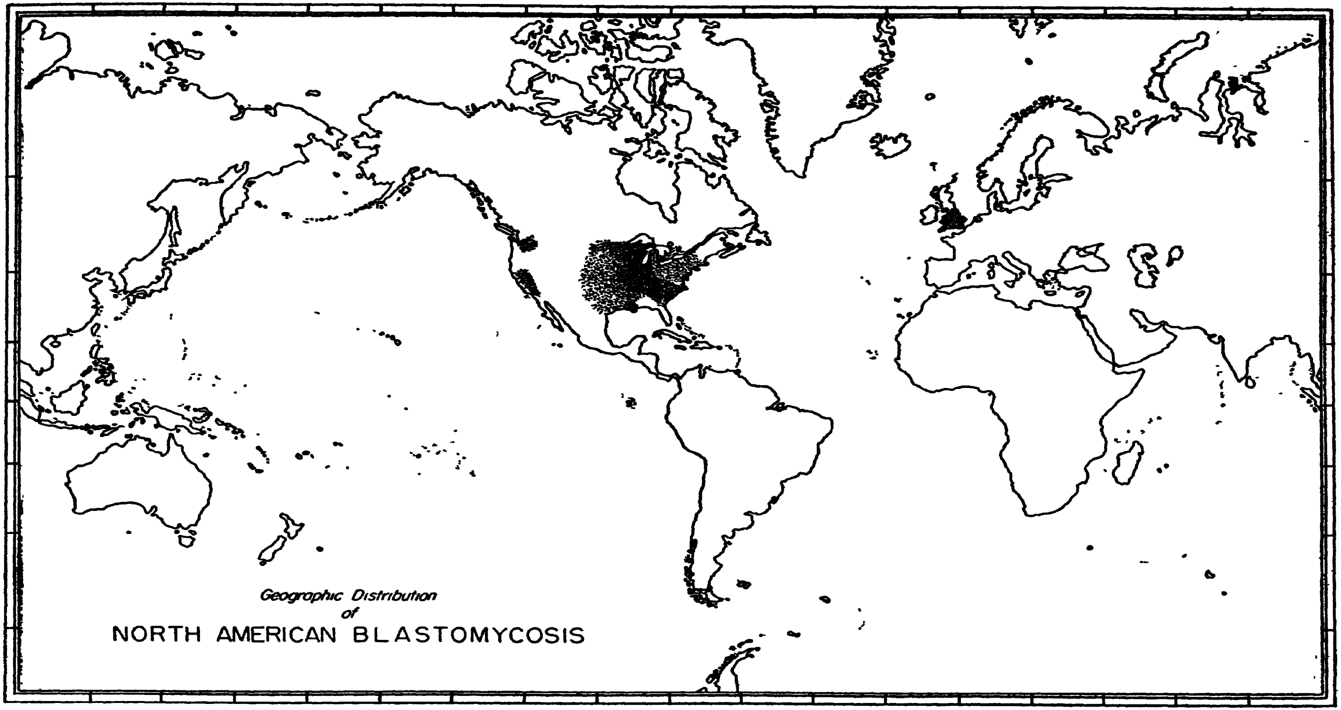
*South American blastomycosis.* — As far as is known at present, infection with *Blastomyces brasiliensis* (*Paracoccidioides brasiliensis*) is limited to South America where it has been reported from Venezuela, Brazil, Argentina, Peru, and Paraguay (*see* Fig. 7). The prevalence of the infection in certain of these areas is high as judged by the large numbers of cases which have been reported by individual investigators studying the disease.

As in North American blastomycosis, the majority of patients are males and the infection is found most frequently in manual laborers whose occupation requires direct contact of the skin with vegetative material.

**Miscellaneous Fungus Infections:** — *Nocardiosis* (*actinomycosis*). — The aerobic species of the family *Actinomycetaceae* now are called *Nocardia.* — The disease produced by these aerobic organisms is indistinguishable from actinomycosis caused by *A. bovis,* but the organisms occur in nature throughout the world and must, therefore, be included among the mycoses of exogenous origin. Pathogenic *Nocardia* have been recovered in a few instances from the soil.

*Geotrichosis.* — Several species of the genus *Geotrichum* occur normally in the mouth and intestinal tract of man. They produce a disease of the oral mucous membranes which simulates thrush; they also produce bronchitis, pulmonary infections and possibly, under rare conditions, infections of the intestinal mucosa. Geotrichosis is mild, as a rule, but may be confused with North American blastomycosis.

*Aspergillosis, mucormycosis, and penicilliosis.* — These molds are present in abundance in nature in all parts of the world. They produce


Geographic Distribution
of
NORTH AMERICAN BLASTOMYCOSIS

— FIGURE 6 —

disease frequently in the ears, nose, and sinuses, and in the lungs and other organs occasionally. In France, squab feeders and fur cleaners not infrequently develop aspergillosis of the lungs from the inhalation of large numbers of these spores. In the wheat-growing regions of the United States, *Aspergillus* occurs in abundance about the wheat threshers and may be inhaled with the dust into the lungs, giving rise to a peculiar type of miliary calcification (SCHNEIDER, 1930).

*Maduromycosis.* — Maduromycosis is an example of an exogenous infection of world-wide distribution. Twenty-two species of fungi belonging to nine genera have been isolated from the lesions of maduromycosis. The aerobic *Actinomyces,* or *Nocardia,* and *Monosporium apiospermum* are encountered most frequently. The disease occurs most often in tropical and subtropical zones when the feet come into direct contact with the soil. Instances have been reported from India, Africa, Europe, South America, Mexico, Canada, and the United States where it has been recognized in Massachusetts, Indiana, Iowa, Minnesota, Maryland, North Carolina, Georgia, Arizona, California, and Texas.

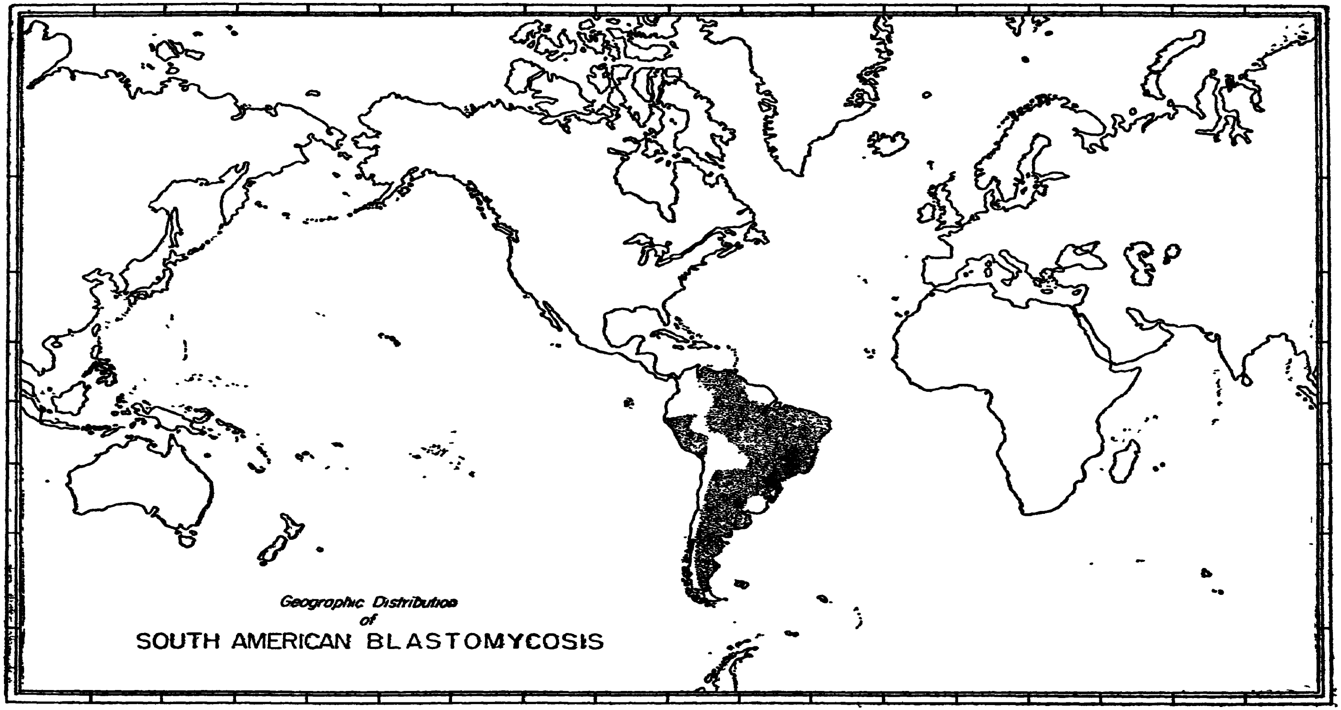
*Rhinosporidiosis.* — Rhinosporidiosis is an exogenous infection caused by *Rhinosporidium Seeberi.* It occurs with great frequency in India and Ceylon; sporadically in Argentina, Paraguay, Brazil, South Africa, the Philippines, and the Malay States; and occasionally in Persia, Italy, England, Scotland, and the United States. Thirteen cases have been found in the United States.

Most of the patients give a history of swimming or diving in stagnant water. The lesions are pedunculated or sessile polyps which occur on the mucous membranes of the nasopharynx or the conjunctivae of the eye. Death may occur from laryngeal obstruction or secondary infection, but the disease does not spread through the body.

*Coniosporiosis.* — Coniosporiosis is a relatively rare disease. It was found in northern Michigan in lumberjacks who were engaged in peeling the bark from maple logs (TOWEY, SWEANY, and HURON, 1932). Clouds of spores of *Coniosporium corticale* were inhaled by the workers, and severe pulmonary symptoms developed with bronchopneumonic patches in the lungs. The patients recovered slowly after a period of weeks or months; on re-exposure to the spores, they immediately relapsed. These patients gave positive skin tests to an extract of the spores, and experimental work in guinea pigs suggests that the disease is primarily an allergic manifestation and not a true progressive infection.

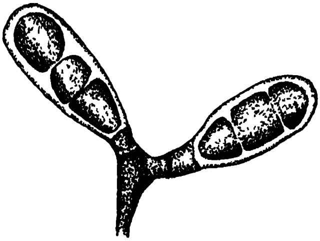
*Literature Cited: —*

CARRIÓN, A. L. Puerto Rico Jour. Public Health and Trop. Med., *11:* 663, 1936.
CONANT, N. F., D. S. MARTIN, D. T. SMITH, R. D. BAKER and J. L. CALLAWAY. Manual of Clinical Mycology. W. B. Saunders Co., Philadelphia, 1944.
DOWLING, G. B. and R. R. ELSWORTHY. Proc. Roy. Soc. Med., *19,* dermatologic section 4, 1925.


Geographic Distribution
of
SOUTH AMERICAN BLASTOMYCOSIS

— FIGURE 7 —

Du Toit, C. J. Proc. Transvaal Mine Med. Officers Assoc., *22:* 111, 1942.
Emmons, C. W. Public Health Repts., *58:* 1, 1943.
Marcuse, P. and J. Karotkin. Texas State Jour. Med., *39:* 534, 1944.
Martin, D. S. Am J. Trop. Med., *18:* 421, 1938.
Palmer, C. E. Public Health Repts., *60:* 513, 1945.
Sanfelice, F. Centrlbl. f. Bakt. u. Parasit., *18:* 521, 1895.
Schneider, L. V. Am. Rev. of Tuberculosis, *22:* 267, 1930.
Slack, J. Jour. Bact., *43:* 193, 1942.
Smith, C. E. Am. J. Public Health, *30:* 600, 1940.
Towey, J. W., H. C. Sweany and W. H. Huron. J. A. M. A., *99:* 453, 1932.
*99:* 453, 1932.
Tschernjawski, J. Arch. f. Derm. u. Syph., *157:* 196, 1929.



### *Chapter 9*

# NUTRITION AND METABOLISM OF PATHOGENIC FUNGI

*by*

WALTER J. NICKERSON *and* JOHN W. WILLIAMS

**Introduction:**— It is a general premise that the more known about a situation or an object, the more possible it becomes to cope with the situation or object. Such may be said for the fungi causing disease in man. It is a matter of considerable importance and interest to have experimental data on the nutrition and metabolism of these organisms. Such information will permit comparison with the numerous non-pathogenic forms that have been, in many cases, very thoroughly studied. The comparisons may lead us to some hypotheses, admitting of experimental test, concerning the metabolic reactions of the pathogen that might distinguish it from related non-pathogenic fungi. Some insight may thus be gained into the necessity, cause, or "reason" for the parasitic life of the pathogen and further progress towards the specific prevention or treatment of the disease caused by the pathogen.

Among workers concerned with the pathogenic fungi one aim has been the isolation of an organism for purposes of identification and establishing proof of its pathogenicity. Any medium repeatedly permitting good growth of an organism was satisfactory for these purposes. Such work will of course continue to occupy many workers in the field, but attention from an increasing number is being directed towards obtaining chemically defined media for each organism in order to elucidate the nutritional requirements (inorganic as well as organic) of each pathogen. Knowledge of the requirements of a pathogen from a host may permit attention to be focussed on means of preventing the pathogen from obtaining its requirements. Growth in chemically defined media permits one to experiment in search of inhibitory compounds without complications from the buffering, adsorbing, and protective effects of proteins, colloids, and unknown substances generally. The leads thus gained may be checked by *in vitro* experiments in the presence of natural products, thereby eliminating from *in vivo* testing all but the more promising substances.

From the work of KNIGHT (1936) and LWOFF (1938) there has developed a general concept that the growth factor requirements of microorganisms are very much the same, and the demand by any individual for an exogenous supply of a given factor reflects a loss of synthetic capacity on its part. As stated by TATUM (1944), "One of the main contributions of this concept is that the losses in synthetic capacities are frequently correlated with an adaptation or modification to more and more complex environments as far as vitamin supplies are concerned." An essential metabolite for which an organism loses synthetic ability must then be obtained from some exogenous source, and an animal (or plant) could be such a source. It is evident that any further modification that is inheritable (powers of synthesis once lost are apparently rarely regained) in the metabolic nature of the microorganism increasing its invasive ability will have survival value for the pathogen. The work of LWOFF referred to above was with the nutrition of protozoa and that of KNIGHT with bacteria. It has been considered (*see* TATUM, 1944) that the fungi also fit in with this concept. So far as the plant pathogens are concerned, it seems clear that most of these have absolute deficiencies for one or more growth factors (ROBBINS and KAVANAGH, 1942). But it is not clear that there is any correlation between the organic growth factor requirements of the fungi pathogenic for man and their tendency towards a pathogenic existence.

As will be shown, some of even the most virulent pathogenic fungi may be grown on very simple chemically defined media. The absence of organic growth factor requirements at least makes permissible an independent non-exacting existence in nature as a saprophyte for such forms as *Coccidioides*. Indeed, all evidence indicates (*see* HENRICI, 1940) that the fungi causing systemic infections have a saprophytic existence in nature. Such a situation may require a shift of emphasis away from some of the implications of the growth factor-pathogenicity work that has been so valuable with bacteria and protozoa and previously assumed to hold for the fungi pathogenic for man; corresponding increases in emphasis may be placed on other metabolic characteristics of the pathogenic fungi. For instance, the real requirement by many species of relatively high nutritional levels of certain heavy metals might bear closer scrutiny.

**Growth on Natural Products and Complex Media:**— Since the site of attack of the dermatophytes is the hair, nails, and epidermis of animals, it was natural that interest was early developed in the ability of these fungi to grow *in vitro* on such products. Growing cultures of species of *Trichophyton* were found by ROBERTS (1894) to attack, to a varying degree, hair of human and animal origin; some species destroyed the cuticle and cortex simultaneously, others worked on the cortex first and but little on the cuticle. MACFAYDEN (1894) was able to grow *Trichophyton tonsurans* on purified keratin obtained from quills; the keratin was made more soluble in strong alkali by the action of the fungus, but MACFAYDEN did not demonstrate a definite enzyme acting on keratin. NANNIZZI (1926) obtained luxuriant growths of dermatophytes on hair and on feathers and suggested that the organisms are able to hydrolyse keratin. But TATE (1929*b*), employing acetone powder preparations of enzymes from several dermatophytes, was unable to observe any hydrolysis of purified keratin with such preparations.

*Keratin.*—There are a variety of keratins from animal sources, but available evidence indicates a considerable degree of similarity among them. The few immunological studies that have been carried out indicate little species specificity among keratins; for example, the lens protein from the eye of a given animal will react with this source of keratin from widely diverse species of animals (*see* LANDSTEINER, 1945). Keratins are characterized by their chemical stability; BLOCK and VICKERY (1931) have emphasized this in their definition, "A keratin is a protein which is resistant to digestion by pepsin and trypsin, which is insoluble in dilute acids and alkalies, in water and in organic solvents, and which, on acid hydrolysis, yields such quantities of histidine, lysine, and arginine that the molecular ratios of these amino acids are respectively approximately 1:4:12." Many keratins, human hair notably, are also remarkable for their high content of the sulfur containing amino acid, cystine; human hair may contain up to 14% of this compound. The resistance of keratins to attack by pepsin and trypsin has called forth theories to explain their stability; SSADIKOW (1926) has postulated different types of linkages between the constituent amino acids from what may occur in other classes of proteins. No enzyme has yet been found that actually hydrolyzes a keratin, but LINDERSTRÖM-LANG and DUSPIVA (1935*a* and *b*) have shown that an enzyme in the intestine of the clothes moth readily digests a reduction product of keratin. Sulfhydryl compounds secreted into the intestine of the clothes moth bring about the reduction of the keratin. It may well be that the action of the dermatophytes on keratin is not through primary enzymatic digestion but secondarily by enzymatic attack on a reduction product, as shown for the clothes moth.

In the preparation of keratin for experimental purposes, it appears from the work of COHEN (1944) that grinding to a powder is contraindicated since the process was shown to split the disulfide linkages, other linkages, and to destroy tryptophane. GODDARD and MICHAELIS (1934, 1935) were able to reduce keratin by splitting the disulfide bonds with the formation of sulfhydryl groups to yield a product they called kerateine. The product of reduction of wool by thioglycolic acid was found by ROUTH and LEWIS (1938) to be digested by both trypsin and pepsin; and the alkali solubility of reduced wool is much greater than that of untreated wool (PATTERSON *et al.,* 1941). JONES and MECHAM (1943) have described a combination method of reduction and dispersion (by urea, acetamide, etc.) for keratins which permits the preparation of a product with a minimum of hydrolysis at neutral *p*H and at as low as 40° C.; they believe the polypeptide chains to be relatively unaltered after this treatment. In light of this recent work it is interesting to recall the early findings of MACFAYDEN cited previously on the increase in solubility of keratin in alkali following the action of *T. tonsurans*. He may have been witnessing an example of increased dispersion following reduction.

*Extractions and hydrolyzates of hair and skin.* — J. W. WILLIAMS (1934*a*) found *Microsporum Audouini* made an appreciable growth in 7 days in distilled water to which hair from a child had been added; with hair from an adult, there was no appreciable growth before 30 days or more. It was pointed out that this is of interest since the pathogenicity of *M. Audouini* is manifest only before adolescence. WILLIAMS (1934*b*) subjected hair from pre-adolescents to continuous ether extraction for 24 hours. Hair with the ether-soluble fraction removed was then added to distilled water and tested as a culture medium with several pathogenic fungi. *Trichophyton* (*Achorion*) *Schoenleinii* was found to grow upon the ether-extracted hair but would not grow upon untreated hair. Many of the fungi examined grew as rapidly on the extracted-hair medium as on the conventional Sabouraud medium; evidently the hair of children is an excellent culture medium for these forms. *Blastomyces dermatitidis* (*Endomyces capsulatus*) grew only on untreated hair; possibly some essential growth factor was removed in the ether extraction process. In comparison studies on ether-extracted hair from adults, WILLIAMS (1935) found again that *Blastomyces dermatitidis,* as well as *Monosporium apiospermum* (*Indiella americana*), showed only scant growth on the extracted hair after 18 days' incubation, though each of these organisms grew well on plain hair. In the other direction, *T. Schoenleinii* was conceivably inhibited by some substance, removable in the ether extraction process, since it grew well on extracted hair within 3 days, but showed only scant growth in 10 days on untreated hair—only slightly better growth than reported earlier on untreated hair from children.

Employing acid hydrolyzates of skin or of hair in place of peptone in culture media, WILLIAMS (1936) obtained good growth with several pathogenic fungi. The growth was of a subsurface type, however, not on the surface as usually obtained with solid media. Similar encouragement of a subsurface type of growth was obtained when peptone was replaced by a combination of alkaline-hydrolyzate of hair (cysteine-cystine thus removed) and cysteine-cystine, in a dextrose agar medium. ROBBINS and MA (1945) also observed that growth of *T. mentagrophytes* with cystine as the sole source of nitrogen was poor and markedly subsurface in character.

*Cellulosic materials.* — It has been stated (GAGE, 1930; GOULD, 1931), but not with the support of experimental data, that species of dermatophytes may grow on cotton; BONAR and DREYER (1932) could find no evidence that representatives of this group could attack cellulosic materials. The latter authors also examined the ability of new sound wood, including oak, Douglas fir, and pine, to support the growth of species of dermatophytes. Blocks of wood, after being moistened with distilled water, were sterilized in flasks and inoculated; after 6 months' incubation at a favorable temperature and relative humidity there was no detectable development of any species. With old weathered wood similarly handled, *T. mentagrophytes* showed appreciable development in 3 out of 12 cultures; viable fungi, however, could be recovered even from the flasks without appreciable growth. Floor boards from showers accumulate a mixture of organic materials in a short time; such material, after sterilization, supported a luxuriant growth of species of dermatophytes.

**Nitrogen Nutrition**:—Although considerable has been written since the time of Sabouraud on the ingredients for culture media for the pathogenic fungi (with regard to dermatophytes especially), little information has been available until recently on the specific nitrogen requirements of any of the pathogens. Peptone has been the source of nitrogen commonly employed in culture media for the pathogenic fungi; peptone is a complex mixture of widely variable composition (witness the many papers on the cultural differences obtained using different peptones). Peptones (not especially purified) contain assorted inorganic compounds in varying amounts, inconstant amounts of amino acids and protein split products, and varying amounts of substances with growth nutrilite functions. Since it is desirable to know, in terms of definite chemical substances, what will satisfy the nitrogen requirements of an organism, it is clear that more definitely characterized substances must be used.

TABLE 1.—*Comparison of growth of normal and pleomorphic strains of T. mentagrophytes with casein hydrolyzate or $NH_4NO_3$ as the sole source of nitrogen; cultures incubated 14 days at 35° C. in liquid media (from* ROBBINS and MA, 1945):—

| ADDITION PER FLASK CONTAINING 25 ML. BASAL SOLUTION* | AVERAGE DRY WT. (MG.) NORMAL | PLEOMORPHIC |
|---|---|---|
| No nitrogen | 0.0 | 0.4 |
| 10 mg. casein hydrolyzate | 15.1 | 36.8 |
| 2 mg. casein hydrolyzate | 3.8 | 7.4 |
| 1 mg. casein hydrolyzate | 1.6 | 3.6 |
| 10 mg. $NH_4NO_3$ | 0.0 | 5.4 |
| 5 mg. $NH_4NO_3$ | 0.0 | 2.5 |
| 1 mg. $NH_4NO_3$ | 0.0 | 0.9 |
| After 10 days' incubation with 2.0 g./liter asparagine included in basal medium | | |
| 10 mg. casein hydrolyzate | 17.3 | 49.6 |
| 1 mg. casein hydrolyzate | 1.8 | 27.7 |
| 25 mg. peptone | 10.8 | 47.1 |
| None | 0.1 | 11.4 |

* Basal solution contained per liter 1.5 g $KH_2PO_4$, 0.5 g. $MgSO_4 \cdot 7H_2O$, and 50.0 g. dextrose with a mineral supplement added (in p. p. m.): 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn.

*Nutrition of dermatophytes.* — One of the first attempts to learn of the nitrogen nutritional requirements of a pathogenic fungus was the careful investigation of MOSHER, SAUNDERS, KINGERY and R. J. WILLIAMS (1936) on *Trichophyton mentagrophytes* (*interdigitale*). These authors found that the fungus would not grow in a medium with only inorganic nitrogen; the organism could not grow in a synthetic medium unless it were supplied with amino acids. There was no evidence for the indispensability of any single amino acid, although leucine seemed to fall nearly in the indispensable category, while asparagine and threonine promoted growth considerably. A varied assortment of amino acids was found to be superior to any single amino acid or group of three or four. There was evidence from color tests on the media deficient in tryptophane and tyrosine wherein the fungus had grown that these amino acids (and therefore possibly others) had been synthesized by the fungus. Though growth with a few amino acids was slower and less extensive than on a casein digest, the fungus was evidently able to modify and synthesize the requisite units for its protein synthesis.

The nitrogen nutrition of *T. mentagrophytes* was again investigated by ROBBINS and MA (1945) in an extensive series of experiments. The findings of MOSHER *et al.* were mainly confirmed. Inorganic nitrogen as $NH_4NO_3$ was found to be almost completely unavailable to this organism (*see* Table 1) and, while no single amino acid was found to be indispensable, no single amino acid promoted growth as well as did a suitable mixture of amino acids. The fungus could transform a single amino acid such as asparagine into all of the various amino acids necessary for the synthesis of its proteins. Apparently the value of protein hydrolyzates lies in the fact that the fungus can assemble the amino acids into its requisite proteins faster than it can transform any single amino acid into such protein. The near essentiality observed by MOSHER *et al.* for leucine was not apparent in the experiments of ROBBINS and MA. Leucine did promote growth in the early stages (through 7 days' incubation) better than any other amino acid but was not outstanding after that.

*Nutrition of variant strains.* — A pleomorphic strain that developed from the isolate of *T. mentagrophytes* used by ROBBINS and MA was found by them to differ physiologically as well as morphologically from the parent or "normal" strain. The pleomorphic strain was observed to have a higher rate of growth than the parent when supplied with asparagine or casein hydrolyzate and was able to utilize ammonium nitrate as the sole source of nitrogen (*see* Table 1). Growth with $NH_4NO_3$ was not so rapid though as with casein hydrolyzate. ROBBINS and MA speculated in an interesting fashion on the apparent similarities between the sudden appearance of a rapidly growing variant in a culture and of a rapidly growing cell in a tissue. They did not remark, however, of the importance to be attached to what is apparently a gain in synthetic abilities by a fungus through mutation. It would seem that the ability to assemble cell constituents from $NH_4NO_3$ represents a distinct gain in synthetic mechanisms and it is most unusual in light of recent genetic studies on fungi (*see* TATUM, 1944; BEADLE, 1945) for mutations to be other than losses of synthetic ability.

The mutants that can appear suddenly in cultures of dermatophytes appear to be permanent in nature; the mutant designated as the "pleomorphic" form *sensu* SABOURAUD is a white, fluffy, non-sporulating growth which appears very commonly in old cultures of most species (it is probably this form which ROBBINS and MA used). In an extensive study of variation in dermatophytes, COUSINS (1936) isolated mutants from 4 species: *E. floccosum, T. mentagrophytes, M. canis,* and *M. gypseum.* Among 4 mutants obtained from *Microsporum canis* one was the pleomorphic form and notable for its rapid growth, while a second one was slow growing and glabrous. No fewer than 20 stable mutants were obtained from *M. gypseum;* the pathogenicity of 3 of these was compared with that of the parent strain by inoculations into guinea pigs—the variants were fully as pathogenic as the parent. COUSINS obtained indicative evidence that the production of these variants possibly results from irregularities in nuclear behavior, possibly genetic rearrangements; four identical mutants were isolated from 4 separate normal strains of a species and some variants were isolated repeatedly from their respective normal strains. In addition to the possible gains in synthetic ability in the variants already mentioned (growth rate and $NH_4NO_3$ utilization) COUSINS found variants notable for their pigment production. It may be that these forms would repay a thorough investigation; there would not be the complications attendant with some of the few other instances where gains in synthetic ability have apparently arisen by mutation (utilization of ammonia by bacteria, KNIGHT, 1936; growth factor synthesis by yeasts, LEONIAN and LILLY, 1943; and phage resistance in *E. coli,* LURIA and DELBRUCK, 1943). For in cultures of single cell organ-

TABLE 2. — *Changes in the composition of a calcium caseinate medium caused by the growth of T. mentagrophytes for 30 days at 25° C.; values in mg./100 cc. of media (from* GODDARD, 1934) : —

| NITROGEN FRACTION | NO SUGAR | | GLUCOSE PRESENT | |
|---|---|---|---|---|
| | MEDIUM | CULTURE | MEDIUM | CULTURE |
| Ammonia | 0.1 | 10.3 | 0.1 | 15.7 |
| Amide | 3.7 | 15.5 | 2.9 | 16.1 |
| Peptide | 0.0 | 81.7 | 9.5 | 62.1 |
| Non-protein | 4.5 | 107.6 | 9.5 | 93.9 |
| Protein | 130.5 | 22.9 | 123.8 | 39.4 |
| Total nitrogen | 135.0 | ... | 133.3 | ... |
| Glucose | None | None | 1384. | 1298. |

isms the possibility of selection of one exceptional individual in a population (within the normal distribution limits of the population) is most difficult to rule out (discussed further in Nickerson, 1943).

*Metabolism of proteins and peptones by dermatophytes.* — Glucose was found by Goddard (1934) to exert a "sparing" effect on the hydrolysis of calcium caseinate by cultures of *T. mentagrophytes* (*interdigitale*) as shown in Table 2. In his study of the metabolism of non-pleomorphic strains, Goddard investigated some of the changes taking place in peptone and in peptone-glucose media during growth of the fungus. With a 1% solution of Chassaing peptone at 25° C. there occurred a transient increase in amino nitrogen, followed by a decrease with a simultaneous increase in ammonia and rise in alkalinity (*see* Table 3). In glucose-peptone cultures similar events occurred whether the concentration was 1% or 4% glucose (*see* Fig. 1). The

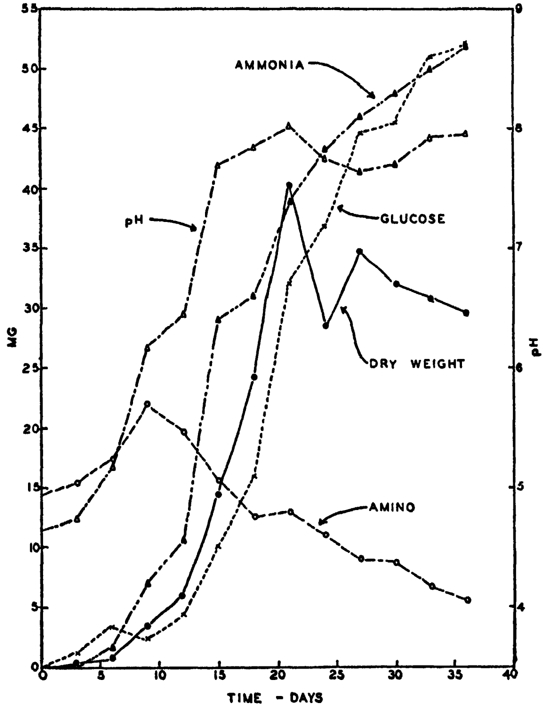


Figure 1. — *Changes in a glucose-peptone culture medium during growth of Trichophyton mentagrophytes.*—Ordinate at left expressed in mg. of substance produced or consumed; multiply values given for amino-nitrogen and ammonia-nitrogen produced (1×), dry weight of fungus produced (10×), glucose consumed (20×)—*e.g.* 20 mg. $NH_3$, 200 mg. dry wt. of fungus, 400 mg. glucose. (*From* Goddard, 1934.)

presence of glucose had no effect on the increase in alkalinity of the cultures, nor on the increase in ammonia. Evidently the increasing alkalinity in cultures of dermatophytes can be generally attributed to the production of ammonia from amino acids by what is probably an oxidative deamination process.

*Nitrogen nutrition of Coccidioides immitis.* — Of several nitrogen sources tested by BAKER and SMITH (1942), all were suitable as the sole source of nitrogen for *C. immitis* when added to a basal medium containing salts, and 1.5% glucose with the exception of nitrite (added as $KNO_2$). Growth was only fair with nitrate (added as $KNO_3$) but heavy growth occurred with $NH_4Cl$, urea, acetamide, asparagine, glycine, alanine, glutamic acid, tyrosine, cystine, and peptone. These findings (which held for 15 strains) were in good agreement with those of STEWART and MEYER (1938) with a possible exception in regard to the utilization of nitrate, which the latter authors classed as supporting only poor growth, but this may be only a relative matter. As with the carbon-nutrition of this organism, requirements with respect to the nitrogen source are singularly non-exacting for a serious pathogen and at least make it permissible that *C. immitis* may exist as a saprophyte in nature (*see* the chapter on *Coccidioides* by EMMONS in this volume). *C. immitis* is no more exacting in its requirements for growth than the common contaminant *Aspergillus niger.* Traditional concepts of the exacting nature of serious patho-

TABLE 3. — *Changes in composition of the medium during the growth of T. mentagrophytes on 1% Chassaing peptone at 25° C.; values in mg./100 cc.* (*from* GODDARD, 1934) : —

| AGE OF CULTURE (DAYS) | *p*H | NITROGEN FRACTION | | | | | | | | WEIGHT OF FUNGUS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AMMONIA | AMIDE | PEPTIDE | PROTEIN | NON-PROTEIN | TOTAL | GLUCOSE | |
| 0.925 percent glucose | | | | | | | | | |
| 0 | 4.72 | 5.19 | 25.4 | 61.1 | 34.9 | 91.7 | 126.6 | 925 | 0 |
| 7 | 4.94 | 13.92 | 28.7 | 63.6 | 14.5 | 106.2 | 121.7 | 866 | 11 |
| 14 | 6.64 | 32.07 | 31.7 | 42 6 | 16.0 | 106.4 | 122.5 | 871 | 63 |
| 21 | 7.78 | 32.39 | 21.1 | 61.2 | 5.0 | 114.5 | 119.6 | 750 | 150 |
| 3.710 percent glucose | | | | | | | | | |
| 0 | 4.70 | 5.07 | 31.3 | 52.3 | 39.5 | 88.8 | 126.5 | 3710 | 0 |
| 7 | 4.85 | 10.06 | 33.5 | 50.8 | 22.6 | 94.7 | 117.3 | 3607 | 11 |
| 14 | 6.88 | 19.19 | 37.6 | 55.3 | 8.4 | 114.2 | 122.1 | 3648 | 71 |
| 21 | 7.78 | 36.24 | 24.7 | 46.3 | 2.8 | 106.9 | 109.7 | 2932 | 347 |
| No glucose added | | | | | | | | | |
| 0 | 5.30 | 4 9 | 19.3 | 65.1 | 35.7 | 89.3 | 124.5 | .... | ... |
| 15 | 6.28 | 8.2 | 21.2 | 77.6 | 15.3 | 106.9 | 122.2 | .... | ... |
| 22 | 6.36 | 8.4 | 20.3 | 81.4 | 15.8 | 110 0 | 125.8 | .... | ... |
| 30 | 8.20 | 46.1 | 19.6 | 59.9 | 0.3 | 125.8 | 126.0 | .... | ... |

gens based on bacteria and protozoa find little support from pathogenic fungi.

**Carbon Nutrition:**—One of the earliest studies of the metabolism of pathogenic fungi was that carried out by VERUJSKY (1887) in DUCLAUX's famous laboratory. *Trichophyton* (*Achorion*) *Schoenleinii* was found to be unable to utilize sugar from any medium but assimilated nitrogenous substances readily. On the other hand, *T. tonsurans* did not invert sucrose but was found to utilize glucose. On a malt medium the ratio for *T. tonsurans* of weight of fungus formed to weight of glucose consumed was 1:2, while the addition of glycerin increased the ratio to 2:3 (in neither case was nitrogen utilization measured, nor was glycerin disappearance estimated). In these experiments, when *T. tonsurans* was grown on impoverished media such as Raulin's medium, oxalic acid was detected as a product; VERUJSKY regarded this as a definitely abnormal product and no subsequent investigation has found this compound as a metabolic product of this species or of any other dermatophyte. BODIN (1899) found *Microsporum canis* (*equinum*) did not utilize sucrose but assimilated glucose, dextrin, and maltose. *Trichophyton mentagrophytes* (*Achorion Quinckeanum*) was found by BODIN (1902) to utilize glucose more readily than lactose or maltose. TATE (1929*a*) reported none of the dermatophytes he studied could utilize sodium acetate, formate, or lactate as a carbon source. MALLINCKRODT-HAUPT (1927, 1933) found species of the dermatophytes could utilize neutral fats as the sole source of carbon if they were of animal origin, but showed little or no growth with vegetable oils. TATE (1929*b*) studied the enzymes

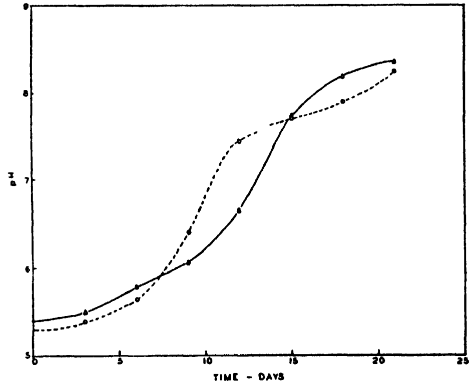


FIGURE 2. — *p*H developed in cultures of *Trichophyton mentagrophytes* grown in peptone (solid line) and in glucose-peptone media. The rise in alkalinity is practically independent of the presence of glucose. (*From* GODDARD, 1934.)

of several of the dermatophytes, using acetone-powder preparations; of the carbohydrases examined, invertase, lactase, zymase, and inulase were absent in all forms. Maltase and diastase were present to a varying degree in all species examined. The general carbohydrase activity appeared to vary inversely with the proteolytic activity, the species with the least trypsin having the greatest carbohydrase activity.

Using freshly isolated non-pleomorphic strains of *Trichophyton mentagrophytes* (*interdigitale*) and *Microsporum canis* (*lanosum*) GODDARD (1934) measured the increase in dry weight of the growth obtained on adding various sugars to a 1% peptone-distilled water medium. *T. mentagrophytes* was found to assimilate glucose, mannose, and galactose, but not lactose. *M. canis* had very similar assimilative abilities but did not utilize either lactose or galactose (*see* Table 4). These findings are in general agreement with those of TATE on the enzymes of dermatophytes, although TATE did not find sucrase in the organisms he studied. It will be noted in Table 3 that the increase in alkalinity in all cultures was independent of the presence of sugar, indicating that the *p*H change resulted from the utilization of the peptone; this is also shown in Figure 2.

TABLE 4.—*Dry weight of Microsporum canis (lanosum) grown at 25° C. in a 1% peptone-distilled water medium with various carbohydrate additions (from* GODDARD, 1934) :—

| CARBOHYDRATE ADDED (1%) | DRY WEIGHT FUNGUS (MG.) | ORIGINAL *p*H | FINAL *p*H | INCUBATION TIME (DAYS) |
|---|---|---|---|---|
| Control (none) | 58.5 | 6.3 | 8.1 | 25 |
| Sucrose | 70.5 | 6.3 | 8.0 | 25 |
| Glucose | 139.0 | 6.1 | 7.6 | 25 |
| Control (none) | 41.0 | 6.84 | 7.77 | 20 |
| Galactose | 32.5 | 6.49 | 7.52 | 20 |
| Lactose | 34.0 | 6.62 | 7.54 | 20 |
| Arabinose | 111.5 | 6.24 | 7.85 | 20 |
| Fructose | 150.0 | 6.44 | 7.87 | 20 |
| Maltose | 155.0 | 6.66 | 7.92 | 20 |
| Glucose | 206.5 | 6.50 | 7.53 | 20 |
| Mannose | 213.5 | 6.44 | 7.29 | 20 |

A high degree of efficiency in assimilation was shown by GODDARD for *T. mentagrophytes*. Some comparative data on filamentous fungi in respect to efficiency of assimilation are shown in Table 5. The peptone supplied *T. mentagrophytes* acted as a carbon source as well as did glucose and this is taken into consideration in the calculation. For the other fungi listed in Table 5 only one utilizable carbon source was present for a given determination. Although *T. mentagrophytes* appears to be comparatively very efficient in its utilization of a carbon source, it nevertheless is a relatively slow-growing fungus, certainly the slowest of the 3 species listed in Table 5. In light of its slight dissimilative ability as determined by measurements of oxygen consumption (NICKERSON and CHADWICK, 1946), one might surmise that energy-yielding reactions are a limiting factor to the growth of this organism.

*Carbon nutrition of Coccidioides immitis.* — Some conflicts appear in the literature concerning the utilization of certain carbon compounds by *C. immitis* for growth, but all authors are in agreement

TABLE 5. — *Comparison of efficiency of assimilation by various filamentous fungi:* —

| ORGANISM | g. CARBON SOURCE USED/g. INCREASE IN DRY WT. | ECONOMIC COEFFICIENT* | CARBON SOURCE | NITROGEN SOURCE | REFERENCE |
|---|---|---|---|---|---|
| *Trichophyton mentagrophytes* ....... | 1 625† | 61.53 | glucose | peptone | 27 |
| *Aspergillus niger* .. | 2.54 | 39.3 | glucose | $NH_4$ lactate | 10 |
| | 2.72 | 36.7 | glucose | asparagine | |
| | 2.78 | 35.9 | glucose | $(NH_4)_2SO_4$ | |
| | 3.41 | 29.3 | glucose | $NaNO_3$ | |
| *Aspergillus niger* .. | ... | 45.87 | sucrose | KCNO | 74 |
| | ... | 44.84 | sucrose | $NH_4Cl$ | |
| | ... | 42.81 | sucrose | $NH_4NO_3$ | |
| | ... | 39.57 | sucrose | urea | |
| | ... | 35.86 | sucrose | $NaNO_3$ | |
| | ... | 19.80 | sucrose | $NaNO_2$ | |
| | ... | 6.72 | sucrose | $HONH_2{\cdot}HCl$ | |
| *Aspergillus niger* .. | ... | 36.52 | mannitol | $NH_4NO_3$ | 74 |
| | ... | 24.35 | mannitol | $NH_4Cl$ | |
| | ... | 23.93 | mannitol | $NaNO_2$ | |
| | ... | 22.94 | mannitol | $NaNO_3$ | |
| *Fusarium lini* ..... | 9.37 | 10.67 | *dl*-alanine | *dl*-alanine | 89 |

* g. increase dry wt./100 g. carbon source used.
† Based on utilization of both glucose and peptone.

that the metabolism is purely oxidative, there being no report of the fermentative decomposition of carbohydrates. In an effort to clarify the situation, BAKER and SMITH (1942) investigated the availability of over 40 carbon compounds for several strains of *C. immitis*. As will be seen in Table 6, a variety of compounds will serve as a suitable carbon source when added to a basal medium containing salts and 0.2% $NH_4Cl$. No special growth factors were found to be necessary. Tubes of media were inoculated with suspensions of chlamydospores and hyphal fragments for each of 15 strains; little strain variation was observed except for slight growths in some of the sucrose and lactose tubes. While the sodium salts of formic, butyric, oxalic, tartaric, gluconic, and citric acids were not utilized, these compounds were not toxic since spores in the inoculation germinated and developed briefly (at the expense of reserve stores within the spore) just as did the controls in the basal medium alone. These results were in agreement with the earlier findings by HIRSCH and BENSON (1927) that lactate forms a suitable substrate and with STEWART and MEYER (1938) who found acetate, but not formate (and amino acids, but not urea), to be suitable as a carbon source. *C. immitis* is thus seen to be non-exacting in regard to its carbon source and to possess no special growth factor requirements.

TABLE 6.—*Suitability of various compounds as the sole carbon source for Coccidioides immitis when added to a basal medium of salts* and 0.2%* $NH_4Cl$*; cultures incubated two weeks at 34° C.; number indicates relative growth (glucose, 4+; basal medium alone, 0) (data from* BAKER *and* SMITH, 1942):—

*Hexoses:*
glucose 4+
fructose 4+
mannose 4+
galactose 4+

*Pentoses:*
xylose + to 3+
arabinose 0

*Disaccharides:*
sucrose 0
lactose 0
maltose 4+
cellobiose 4+
trehalose 4+

*Polysaccharides:*
starch 4+
dextrin 4+
cellulose 0
inulin 4+

*Trisaccharides:*
raffinose + to 2+
melizitose 0

*Glucosides:*
α methyl glucoside 2+ to 3+
salicin 4+
amygdalin 4+

*Alcohols:*
ethanol 4+
glycerol 4+
erythritol 4+
mannitol 4+
sorbitol 4+
*i*-inositol 0

*Fatty acids:*
formate 0
acetate 4+
propionate 2+
butyrate 0
caproate 2+

*Dicarboxylic acids:*
oxalate 0
succinate 4+
tartrate 0
fumarate 3+
malate 3+

*Hydroxy acids:*
lactate 4+
gluconate 0
citrate 0

*Keto acids:*
pyruvate 4+

*Amino acids:*
glycine 2+
alanine 2+ to 3+
cystine +

*Amides:*
urea 0
acetamide 4+
asparagine 2+ to 3+

* Salts in basal medium (in grams per liter): 2.0 g. $KH_2PO_4$, 2.0 g. $K_2HPO_4$, 0.5 g. $MgSO_4$, 0.1 g. $FeCl_3$.

**Mineral Nutrition:**—In a general study of the nutritional requirements of *Trichophyton mentagrophytes* (*interdigitale*), MOSHER *et al.* (1936) examined its mineral requirements, including the "micronutrient" or "trace" elements. Although the nitrogen requirements of this fungus could not be satisfied with ammonium nitrate, it was still found to require the ammonium ion for growth. Other ions in the "macronutrient" class were potassium, magnesium, and calcium. The essential micronutrients were: zinc, iron, copper, manganese, phosphate, and sulfate. Of the ions tested, four seemed to be nonessential for *T. mentagrophytes;* these were thallium (RICHARDS, 1932, has shown the essentiality of thallium for a strain of *Saccharo-*

*myces cerevisiae*), iodide, chloride, and borate. The basal medium contained the following amounts per liter:

| | | | | | |
|---|---|---|---|---|---|
| $(NH_4)_2SO_4$ | 3.00 g. | $FeCl_3$ | 0.10 mg. | KI | 0.10 mg. |
| $KH_2PO_4$ | 4.00 g. | $ZnSO_4$ | 1.00 mg. | $ThCl_3$ | 0.10 mg. |
| $CaCl_2 . 2H_2O$ | 0.10 g. | $MnCl_2$ | 1.00 mg. | $H_3BO_3$ | 1.00 mg. |
| $MgSO_4$ | 0.25 g. | $CuSO_4$ | 0.10 mg. | | |

Suitably supplemented with carbon, nitrogen, and nutrilite sources, this medium permitted excellent growth of *T. mentagrophytes*. When portions of this list were omitted the following effects on growth were observed:

| *Medium* | *Growth* |
|---|---|
| Complete mineral component present | + |
| $KH_2PO_4$, $MgSO_4$, $CaCl_2$, or $(NH_4)_2SO_4$ omitted | — |
| $FeCl_3$, $CuSO_4$, or $MnCl_2$ omitted | — |
| $PO_4$ or $SO_4$ omitted | — |
| $ThCl_3$, KI, and $H_3BO_3$ omitted | + |

The difficulties surrounding investigations of the micronutrient requirements of higher plants (*see* HOAGLAND, 1944) have their counterpart in comparable investigations with fungi. Impurities in even the highest quality chemicals and dissolution of ions from glass culture vessels are possible sources of the ion under investigation and must be carefully watched.

The extensive work of STEINBERG since 1918 (*see* 1939*a, b*) on the mineral nutrition of *Aspergillus niger* has shown the essentiality of iron, zinc, copper, manganese, molybdenum, and gallium for a strain of this mold. There is evidence that molybdenum has an important role in *A. niger* in the reduction of nitrates and nitrites—of critical importance when these compounds were the sole source of nitrogen. His researches have also brought to light the extreme care that must be exercised during such work since the minute amounts of the heavy metal required can easily be supplied as an impurity unless guarded against. Many purification techniques designed to rid nutrient solutions of traces of heavy metals are in use today; some, including the 8-hydroxy quinoline method of WARING and WERKMAN (1943), act by complex formation with heavy metals; others, including STEINBERG'S (1919) use of $CaCO_3$, work by adsorption onto finely particulate surfaces. The latter method is simple and satisfactory. Excess calcium carbonate (15 g./liter) is added to the nutrient solution, autoclaved at 15 lb. for 15 minutes and filtered while hot; the nutrient solution is then distributed as desired and sterilized, care being taken not to reintroduce heavy metals.

In an excellent review of the comparative aspects of heavy metal nutrition of fungi, FOSTER (1939) included a discussion of the relationships between certain of these metals (notably iron, copper, and manganese) and the pigments elaborated by fungi. In particular were the pigments of spores in many fungi shown to be sensitive to the level of mineral nutrition. A study of pigment formation in the dermatophytes using media of known composition with different levels of mineral nutrition would appear profitable and might clear up some of the existing uncertainties regarding pigment formation in this group.

TABLE 7.—*Growth of species of Candida in a chemically defined medium with supplements of growth factors and liver concentrate; values are turbidity units for cultures grown for 72 hours at 25° C. (data of* BURKHOLDER *and* MOYER, 1943) :—

| ORGANISM | NONE | BIOTIN | PYRIDOXIN | PANTOTHENIC ACID | NIACIN | INOSITOL | THIAMIN | PLUS ALL | ALL PLUS LIVER CONCENTRATE |
|---|---|---|---|---|---|---|---|---|---|
| *C. albicans* (No. 416) | 14 | 10 | 148 | 179 | 155 | 139 | 74 | 156 | 210 |
| *C. albicans* (No. 430) | 8 | 8 | 174 | 194 | 190 | 165 | 130 | 205 | 187 |
| *C. albicans* (No. 461) | 6 | 10 | 187 | 192 | 184 | 147 | 156 | 176 | 201 |
| *C. albicans* (No. 462) | 4 | 5 | 224 | 241 | 234 | 202 | 124 | 235 | 281 |
| *C. flareri* (No. 245).. | 0 | 4 | 121 | 113 | 113 | 99 | 98 | 106 | 95 |
| *C. mycotoruloidea* (No. 527) ........ | 0 | 1 | 144 | 156 | 148 | 141 | 61 | 149 | 170 |
| *C. parakrusei* (No. 316) ............. | 0 | 0 | 162 | 167 | 157 | 149 | 157 | 144 | 182 |
| *C. stellatoidea* (No. 523) ............. | 27 | 29 | 185 | 201 | 189 | 179 | 86 | 195 | 206 |
| *C. stellatoidea* (No. 526) ............. | 25 | 36 | 194 | 195 | 193 | 195 | 92 | 195 | 245 |
| *C. tropicalis* (No. 347) ............. | 0 | 0 | 160 | 148 | 72 | 152 | 147 | 134 | 65 |

*Coccidioides immitis.*—ROESSLER *et al.* (1946) grew the fungus submerged in liquid cultures by continuous mechanical agitation; the formation of free spores was thereby encouraged. Growth was abundant in a chemically defined medium with ammonium acetate as the source of nitrogen, glucose, $MgSO_4$, $K_2HPO_4$, $KH_2PO_4$, and $ZnSO_4$. No accessory growth factors were required. Magnesium (used as 0.008M $MgSO_4$) and zinc (0.00003M $ZnSO_4$) were the only cations found to be required for growth.

**Growth Factor Requirements:**—A growing literature on this topic in the nutrition of pathogenic fungi permits some comparisons among the various groups and with related non-pathogenic forms. A recent excellent discussion of the comparative aspects of the growth factor requirements for microorganisms generally is that of KNIGHT (1945), and for plants in general see SCHOPFER (1943). In the genus *Candida,* the one pathogenic species, *C. albicans,* is not distinguished notably from the non-pathogenic species (*see* Table 7). There is as wide variation among different isolates of *C. albicans* as between species of *Candida*. All species are indicated to require biotin for growth, and several have their growth stimulated on the addition of thiamin. *C. albicans* has been shown by VAN NIEL and COHEN (1942) to utilize the thiamin-containing coenzyme cocarboxylase, as evidenced by the accumulation of pyruvic acid with suspensions that had become thiamin deficient through aeration in a non-nutrient medium.

*Thiamin.* — With a few interesting exceptions, growth factor requirements among the filamentous pathogenic fungi are limited mainly to a need for thiamin (*see* Table 8). Some of the dermatophytes, for example strains of *Trichophyton mentagrophytes,* have been shown by several authors to have no growth factor requirements, while *Trichophyton Schoenleinii* (*T. discoides*) has been shown by ROBBINS and MA (1943) to require thiamin and to be unable to synthesize thiamin when supplied with both the pyrimidine and thiazol moieties of thiamin. OYAMA (1937) has described a few instances (*see* Table 8) wherein growth of an organism is not stimulated by thiamin but is stimulated by an extract of rice polishings (a source rich in thiamin).

*Riboflavin.* — There are no reports of any fungi requiring riboflavin for growth, though there is increasing indication that the level of riboflavin nutrition in the cell may vary during the growth cycle in certain organisms and actually be a limiting factor under certain conditions. Such a situation has been shown by DOUDOROFF (1938) with strains of the luminescent bacterium, *Photobacterium phosphorescens;* concentrations of riboflavin sufficient to support growth of some strains were insufficient to permit luminescence in the same strains. MOSHER *et al.* (1936) studied a strain of *T. mentagrophytes* for which riboflavin was stimulating. The concentration level of riboflavin has also been implicated by NICKERSON and THIMANN (1943) in the sexual reproduction of a conjugating yeast, *Zygosaccharomyces acidifaciens,* which has no requirement for added riboflavin during growth but which exhibits a marked increase in the relative number of cells conjugating in cultures to which riboflavin has been added. (The riboflavin stimulation was intensified on the addition of glutaric acid.)

*Pyridoxin.* — Pyridoxin has been implicated in the nutrition of a few of the lower fungi, chiefly through the work of ROBBINS and MA (1942) and of FRIES (1943*a, b*). *Trichophyton Schoenleinii* (*T. discoides*) remains the only pathogenic fungus for which pyridoxin has been reported as a growth factor (ROBBINS, MACKINNON and MA, 1942), though pyridoxin is stimulatory for *Pityrosporum ovale* when oleic acid is present to permit growth (BENHAM, 1941).

*Biotin.* — Biotin has not been shown to be a growth factor for any of the filamentous pathogenic fungi, though it is required by many yeasts (*see* Table 7) and bacteria. SCHOPFER and BLUMER (1942;

Schopfer, 1943) showed that, under certain conditions, biotin can serve as a growth factor for *T. Schoenleinii* (*T. album*). In a medium with glucose, asparagine, $MgSO_4$, and $KH_2PO_4$, only thiamin is re-

Table 8. — *Growth factor requirements of pathogenic fungi grown in a synthetic medium containing purified agar and supplements of growth factors:* —

| ORGANISM | GROWTH FACTOR REQUIREMENTS | GROWTH IN LIVER OR PEPTONE | REFERENCE |
|---|---|---|---|
| *Coccidioides immitis* | none observed | ........ | 1, 66 |
| *Hormodendrum Pedrosoi* | thiamin | somewhat stimulated | 11 |
| *Phialophora verrucosa* | thiamin | ........ | 11 |
| *Sporotrichum Schenckii* | thiamin | not stimulated | 11 |
| *Sporotrichum Schenckii* | thiamin | rice polishings stimulatory | 57 |
| *Trichophyton mentagrophytes* (*T. interdigitale*) | thiamin, inositol, and pantothenate | ........ | 51 |
| *T. mentagrophytes* (No. 514) | none observed | stimulated | 11 |
| *T. mentagrophytes* (No. 517) | none observed | not stimulated | 11 |
| *T. mentagrophytes* | none observed | stimulated | 64 |
| *T. rubrum* | none observed | somewhat stimulated | 11 |
| *T. Sabouraudi* (*T. acuminatum*) | partially deficient for thiamin | not stimulated | 11 |
| *T. Schoenleinii* (*T. discoides*) | thiamin, inositol, and pyridoxin | ........ | 62 |
| *T. Schoenleinii* (*T. album*) | biotin, thiamin, inositol and pyridoxin may stimulate | stimulated | 71 |
| *T. sulfureum* | thiamin | not stimulated | 11 |
| *T. tonsurans* (*T. crateriforme*) | no effect from thiamin | rice polishings stimulatory | 57 |
| *T. violaceum* | thiamin | greatly stimulated | 11 |
| *Microsporum gypseum* (*M. fulvum*) | none observed | yeast extract stimulatory | 88 |
| *Pityrosporum ovale* | oleic acid | thiamin or pyridoxin stimulatory in presence of oleic acid | 3 |
| *Trichosporon Beigelii* | no effect from thiamin | rice polishings stimulatory | 57 |

quired as a growth factor. Though biotin is stimulatory in the early stages of development, growth in controls without biotin was found to overtake cultures supplied with biotin. When $(NH_4)_2SO_4$, rather than asparagine, was used as the nitrogen source, biotin was required even up to advanced stages of development; with ammonium citrate as the nitrogen source, biotin promoted better growth than in controls without biotin over a 24 day period. Apparently the ability of the organism to synthesize biotin varies with the age of the culture and with the nitrogen source.

*Fatty acids.* — The report by BENHAM (1941) of the requirement of oleic acid by *Pityrosporum ovale* for growth in subcultures was the first demonstration of such a need for a fungus. FLEMING (1909) had shown that *Corynebacterium acnes* (the acne bacillus), ordinarily grown only with difficulty, grew well on nutrient agar with 1 to 5% of oleic acid added.

BENHAM discusses her work elsewhere in this volume, but a few figures will be used here for comparative purposes. In a medium with $NH_4NO_3$, salts, and glucose, it was found that 100 mg. oleic acid per liter produced a slight effect with growth increasing up to 10 grams per liter, though growth still was not heavy. Addition of asparagine permitted good growth in 1-2 weeks. In contrast to the concentration levels of oleic acid required by *P. ovale,* it has been found for *Clostridium tetani* by FEENEY, MUELLER and MILLER (1943) that its oleic acid requirement was satisfied optimally at a concentration of only 0.75 mg. oleic acid per liter.

Against these requirements of a fatty acid for growth it has been found by ROTHMAN, SMILJANIC and SHAPIRO (1945) that the hair fat of adults contains free saturated aliphatic fatty acids which inhibit the growth of *Microsporum Audouini* in concentrations of 2 to 10 mg. per liter. Preliminary analyses indicated that the active fraction comprised fatty acids containing 7 to 11 carbon atoms. The fat extracted from children's hair had only 1/5 the fungistatic action of adults' hair fat. It is tempting, of course, to see in this development an explanation of the relative incidence of *Microsporum* infections of the scalp in adults *vs.* children. ROTHMAN (1945) pointed out that the non-hairy skin of adults remains susceptible to infection by *M. Audouini.*

**Physico-Chemical Factors in Growth:**— In addition to the demands an organism may have for growth in regard to carbon and nitrogen sources, minerals, and growth factors, it can be more or less exacting with respect to the *p*H range within which nutrients can be assimilated and its enzymes act. In similar fashion, temperature limits and redox potential limits operate to influence the activity of any protoplasmic system. A consideration of some of these factors and related phenomena for the pathogenic fungi reveals some interesting relationships. The effects of temperature on morphogenesis in some of the fungi that are yeast-like in the body offers a fertile field for further experimentation.

*Hydrogen ion concentration.* — None of the pathogenic fungi is particularly exacting in its *p*H requirements for *in vitro* cultivation. Although great extremes of acidity are not tolerated, each of the pathogens is able to grow over a considerable range of hydrogen ion concentrations. The dermatophytes are possibly the least exacting of the pathogens in this regard. It is possible to culture *Trichophyton mentagrophytes* easily in a range from *p*H 3 to *p*H 10.5. In fact, LEISE and JAMES (1945) have made use of the ability of the dermatophytes to grow well under alkaline conditions in devising a medium buffered to *p*H 10.5 for the isolation of dermatophytes from sources such as shoes, floors, shower baths, etc., where the presence of saprophytes in large numbers has in the past complicated the problem of isolating the slower growing pathogens. There is apparently no sharp *p*H optimum for *T. mentagrophytes,* especially in a good nutrient medium as Table 9 shows; many workers grow the dermatophytes at an initial *p*H of 5.5 where it does well. This *p*H is the same generally found for healthy skin (BLANK, 1939). It has been shown that *p*H 5.5 is the region of lowest rate of respiration for at least two

TABLE 9. — *Effect of pH on the colony diameter and dry weight of mycelium produced by Trichophyton mentagrophytes on agar at three levels of nutrition (from* ROBBINS and MA, 1945) : —

| INITIAL *p*H | FINAL *p*H | AVERAGE DRY WEIGHT (MG.) | COLONY DIAMETER (MM.) |
|---|---|---|---|
| | *Basal medium* | | |
| 4.5 | 4.5 | 0.1 | 6.0 |
| 5.4 | .. | .. | 9.0 |
| 5.5 | 5.4 | 0.2 | .. |
| 6.0 | 5.8 | 0.4 | 10.0 |
| 6.5 | 6.0 | 0.8 | 13.0 |
| | *Basal medium + 1 mg. casein hydrolyzate* | | |
| 4.5 | .. | .. | 30.0 |
| 4.6 | 6.0 | 3.3 | .. |
| 5.5 | 6.2 | 4.0 | 30.0 |
| 6.0 | 6.2 | 4.0 | 30.0 |
| 6.5 | 6.2 | 4.2 | .. |
| 6.6 | .. | .. | 30.0 |
| | *Basal medium + 25 mg. peptone* | | |
| 5.2 | 6.4 | 10.4 | 35.0 |
| 5.5 | 6.4 | 10.3 | 34.0 |
| 5.9 | 6.4 | 11.4 | 34.0 |
| 6.4 | 6.4 | 11.3 | 25.0 |

of the dermatophytes (NICKERSON and CHADWICK, 1946; *see* Chapter 12 of this volume).

MOSHER *et al.* (1936) remarked that the production of bases during growth of *T. mentagrophytes* in Sabouraud's medium changed the *p*H from 5.5 to 8.0, which is a considerable rise in view of the buffering capabilities of peptones and amino acids. ROBBINS and MA (1945) found only a slight increase in alkalinity in 10 days' growth of *T. mentagrophytes* on a peptone medium as shown in Table 9; reference to Figure 1 shows that increases in alkalinity become more pronounced in older cultures—after the buffering effect has been overcome. The *p*H indicator action of the pigments produced by many of the dermatophytes would lead one to believe that the *p*H of the medium is frequently on the alkaline side, since these pigments are reddish under alkaline conditions. The good growth of most of the dermatophytes under conditions as acid as *p*H 3.0 is curious in light of TATE's (1929*b*) failure to find any pepsin-like proteolytic enzyme, though all species investigated possessed trypsin-like enzymes active in alkaline conditions.

In the section on metabolism of proteins and peptones in this chapter, the work of GODDARD (1934) on *T. mentagrophytes* (*interdigitale*) was cited. It is evident from his data that the increase in alkalinity in cultures results from the metabolic production of ammonia by deaminative processes. In Figure 2 the change in *p*H in cultures containing peptone and peptone plus glucose is shown. Together with the decrease in values for volatile acids found in the cultures, these curves indicate the absence of acid production by *T. mentagrophytes* from glucose.

The cessation of growth of *T. mentagrophytes* shown in Figure 1 at *p*H 7.5 may, in some way, be a result of the concentration of the ammonium ion rather than of the *p*H itself, since growth of several of the dermatophytes has been shown (LEISE and JAMES, 1945) to take place readily in media adjusted to *p*H 10.5 with sodium hydroxide. GODDARD (1934) presented *p*H-titration curves on four media commonly employed in culturing pathogenic fungi. The conservation medium of Sabouraud with 4% Chassaing peptone was shown to have considerably greater buffer capacity (resistance to change in *p*H on addition of $H^+$ or $OH^-$ ions) than the Sabouraud proof medium prepared with Chassaing or Bacto peptones; 20 cc. of N/10 NaOH were required to change the *p*H of 100 cc. of the conservation medium (4% Chassaing peptone) from 4.6 to 6.3.

As part of a comparative study on *Histoplasma capsulatum* and morphologically related form-species, HOWELL (1941) investigated the influence of hydrogen ion concentration of the medium on growth and spore formation. Comparing *H. capsulatum* and *Sepedonium chrysospermum* (Bull.) Link—a parasite on other fungi, this strain having been isolated from a *Boletus* sp.—it will be seen in Table 10 that the optimum *p*H for growth for each is about 6.5. At this *p*H *H. capsulatum* produced the most aerial mycelia and achieved the greatest radial growth; aleuriospore production was also maximum. The same relationships held generally for *S. chrysospermum,* but this species maintained good growth to *p*H 5.0 although aerial mycelium and aleuriospore production were poor at the more acid range. The high H-ion concentrations attained by cultures of each species from growth at an initial *p*H of 5.0 indicates the formation of considerable amounts of acid in each case. The metabolic products of neither species appear to have been investigated, however.

TABLE 10. — *Growth and acid production at 22° C. by Histoplasma capsulatum and Sepedonium chrysospermum at different initial hydrogen ion concentrations using a phosphate buffer series and an agar medium of "known" composition** (*from* HOWELL, 1941) : —

| INITIAL *p*H | AGE OF CULTURE (DAYS) | FINAL *p*H | AVERAGE RADIAL GROWTH (DIAM. IN MM.) |
|---|---|---|---|
| | *Histoplasma capsulatum* (M251) | | |
| 5.0 | 31 | 2.8-3.0 | 17.2± 0.3 |
| 5.6 | 31 | 3.0-3.1 | 18.5± 1.5 |
| 6.5 | 31 | 3.8-3.9 | 27.1± 1.6 |
| 7.7 | 31 | 7.1-7.4 | 11.9± 1.7 |
| 8.6 | 31 | 7.9 | 6.0 |
| | *Sepedonium chrysospermum* | | |
| 5.0 | 7 | 1.8-1.9 | 32.7± 1.2 |
| 5.6 | 7 | 2.1-2.3 | 32.3± 1.4 |
| 6.5 | 7 | 3.5-3.6 | 30.9± 1.3 |
| 7.7 | 8 | 5.7 | 31.0± 2.2 |
| 8.6 | 8 | 5.4 | 23.6± 0.2 |

* A modification of that of MOSHER *et al.* (1936): mineral salts, 0.0435%; glucose, 4.25%; $(NH_4)_2SO_4$, 0.3%; agar, 2.5%; trace amounts of leucine, phenylalanine, aspartic acid, inositol, and thiamin.

*Temperature.* — The pathogenic fungi may conveniently be divided into two groups on the basis of the temperature range of growth *in vivo.* The organisms causing superficial skin infections inhabit an environment which when dry reflects atmospheric temperatures to some extent, so that even *in vivo* the dermatophytes are subjected to a considerable range of temperature. Organisms causing deep mycoses are maintained at more constant temperatures. *In vitro* the dermatophytes grow well in a range from 25° C. to 38° C. showing little variation from temperature *per se,* but the slow growth rates necessitate guarding against the drying out of cultures on agar or a retarding of growth by dehydration will occur (though there is a tendency for spore formation to be less abundant above 35° C. as VERUJSKY, 1887, early observed). With some of the fungi causing deep mycoses, the temperature of incubation for *in vitro* growth is quite critical. Characteristic growth forms that develop at 25° C. may be entirely lacking at 37° C. at which temperature the growth *in vitro* may more closely approximate the appearance of the fungus *in vivo,* but be less differentiated and of less value for diagnostic purposes.

*Blastomyces dermatitidis* may develop in culture into one of two quite different forms, depending on the temperature of incubation. At 37° C. the yeast-like cells found in lesions will develop on cultures on blood agar, but at 25° C. a cottony filamentous growth develops on Sabouraud's agar. Many workers had contended that once the mycelial type of growth had been allowed to develop at room temperature the yeast-like growth could not be recovered, but CONANT (1939), confirming CIFERRI and REDAELLI (1935), demonstrated that heavy inoculations from mycelial cultures on blood-agar slants incubated at 37° C. developed typical yeast-like growths. CONANT mentioned that on inoculating yeast-like growths on Sabouraud's agar and incubating at room temperature the growth at first is moist and glabrous, but soon becomes dry and cottony with aerial projections giving the colony a prickly appearance which eventually is replaced by an all over cottony appearance. Numerous conidia were produced in both the prickly and filamentous stages of growth. A fatal infection in mice could be produced by inoculating growths obtained at either room temperature or at 37° C.

TABLE 11.—*Effect of 10 minute exposures at various temperatures on cultures of four species of dermatophytes growing on wool (from* BONAR and DREYER, 1932):—

| TEMPERATURE C. | *T. mentagrophytes* | | *T. Megnini (rosaceum)* | | *E. floccosum* | | *M. canis (lanosum)* | |
|---|---|---|---|---|---|---|---|---|
| | A* | B | A | B | A | B | A | B |
| 70 | 12 | 0 | .. | .. | .. | .. | .. | .. |
| 65 | 20 | 2 | .. | .. | .. | .. | 2 | 0 |
| 60 | 22 | 6 | 12 | 0 | 18 | 0 | 14 | 0 |
| 55 | 24 | 13 | 12 | 0 | 21 | 0 | 20 | 2 |
| 50 | 24 | 23 | 23 | 0 | 21 | 0 | 20 | 4 |
| 45 | 18 | 18 | 23 | 22 | 22 | 12 | 18 | 12 |
| 42 | 6 | 6 | 11 | 11 | 4 | 4 | 12 | 12 |
| *control* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

* A—number of cultures exposed; B—number of subcultures positive.

The effect of temperature on the development in culture of *Histoplasma capsulatum* shows a striking parallel with the case for *Blastomyces.* CONANT (1941) has shown that the saprophytic filamentous form of *H. capsulatum* developed on Sabouraud's agar at room temperature can be converted, without intervention of animal passages, to the yeast-like form found in tissues by cultivation on sealed blood-agar slants at 37° C. CONANT made a strong point in this work that classification of an organism should be based on the phase of the life-cycle exhibiting a spore form; *H. capsulatum* develops characteristic tuberculate chlamydospores on all media at 25° C., but develops only a yeast-like stage at 37° C.

*Heat resistance of dermatophytes.* — In connection with an extensive study on the public health aspects of dermatophyte infections, BONAR and DREYER (1932) examined the effects of different temperatures on four species growing on various natural products. As will be seen in Table 11, *Trichophyton mentagrophytes* was the most resistant to heat of the species examined; spores of this organism were also more resistant than were spores of the other species studied. Although an exposure to 75° C. for 10 minutes killed all the spores, approximately 25% of the spores of *T. mentagrophytes* germinated after 10 minutes at 70° C. With cultures growing on hair, *T. mentagrophytes* proved again the most resistant of the four species, but no subcultures could be obtained after a 10 minute exposure of cultures to 55° C., with only 60% of the subcultures showing growth after being held the same length of time at 50° C. WEIDMAN (1927) also found *T. mentagrophytes* (*interdigitale*) the most heat resistant of the dermatophytes he studied. Comparing Tables 11 and 12 it will be seen that the skin scales in which mycelia of *Epidermophyton floccosum* were imbedded afforded some protection against the lethal effects of elevated temperatures. BONAR and DREYER emphasized that infectious fungus material imbedded in hairs or in skin scales is enclosed in structures highly impermeable to aqueous solution. They also demonstrated markedly increased resistance by mycelia in skin scales to chemical disinfectants as compared with the resistance of spores.

TABLE 12. — *Effect of 10 minute exposures at various temperatures on mycelia of Epidermophyton floccosum encased in skin scales (from* BONAR and DREYER, 1932) : —

| TEMPERATURE C. | Number of scales exposed | Number of scales giving positive cultures |
|---|---|---|
| 70 | 20 | 0 |
| 60 | 60 | 0 |
| 55 | 40 | 0 |
| 50 | 70 | 22 |
| control | 70 | 50 |

*Moisture requirements of dermatophytes.* — In an important study, DAVIDSON and GREGORY (1934) demonstrated that the highly differentiated spore forms commonly found in cultures growing on media also developed from infected hairs and skin scales detached from the host and maintained by *in situ* culture. Hairs naturally infected with *Microsporum Audouini, M. canis* (*felineum*), or *Trichophyton mentagrophytes* (*gypseum*) and skin scales containing *Tricho-*

*phyton* (*Achorion*) *Schoenleinii* were placed without added nutrient in van Tieghem cells maintained at different relative humidities. While parasitizing an animal body, these fungi produce only hyphae or thallospores but under suitable conditions the *in situ* cultures produced the highly differentiated structures characteristic of the species when grown on nutrient media. As the relative humidity decreased from a completely water saturated atmosphere to 97% R. H. there was an increase in the time required for the fungi contained in the infected hairs or skin scales to "germinate" or show signs of growth. As shown in Table 13, a relative humidity as high as 95.8% was insufficient to permit the growth of cultures of *M. Audouini in situ,* while the radial growth of *M. canis* decreased markedly below 97% R. H. The lowest R. H. at which the growth of *T. mentagrophytes* and *T. Schoenleinii* was observed under these conditions was 97% and 93.3% R. H. respectively.

TABLE 13. — *Effect of different relative humidities on two species of dermatophytes (from* DAVIDSON and GREGORY, 1934) : —

|  | *Microsporum Audouini* | *Microsporum canis* |  |
|---|---|---|---|
| RELATIVE HUMIDITY (%) | Germination from infected hairs (time in days) | Germination (time in days) | Mean daily radial growth in μ 2nd and 3rd days |
| 100 | 1 | 1 | 1,000 |
| 99 | 2 | 1 | 870 |
| 98 | 5 | 1 | 500 |
| 97 | 10 | 2 | 350 |
| 95.8 | none | 4 | 50 |

The values presented place these fungi at the upper end of the scale of minimum relative humidity for growth in comparison with the requirements of other fungi. BONNER (1945) listed the range of minimum relative humidity for 58 fungi at from 70% to 98% with a fairly even distribution of species over that range. (In this paper BONNER describes an elegant method, based in part on that of GALLOWAY, 1935, for studying the influence of relative humidity on spore germination, and reports the finding of a strain of *Aspergillus niger* for which the optimum relative humidity was not saturation, but 93% R. H.) It is possible, therefore, that a slight reduction in the relative humidity from saturation is sufficient to stop the growth and development of several dermatophytes. The beneficial results on the incidence and severity of foot infections prompted by the routine wearing of open-toed sandals as found in a large-scale test with nearly 2500 soldiers was attributed by NICKERSON, IRVING and MEHMERT (1945) largely to the free access of air and consequent removal of saturated water vapor from the vicinity of the feet.

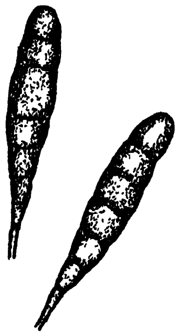
*Radiations.*—It appears to be a matter of individual preference in each laboratory whether cultures of pathogenic fungi are grown in the light or the absence thereof; no incontrovertible evidence has accumulated to make a real brief for either procedure. The use of x-rays in therapy of fungus diseases and the use of ultraviolet in detection of fungus infections of the hair and scalp are well known. Few intensive studies of the effects of radiations on these organisms have been conducted, but one excellent study by HOLLAENDER and EMMONS (1939) and EMMONS and HOLLAENDER (1939) deserves attention. They found monochromatic ultraviolet light between 2537 and 2650 Å to be fungicidal for spores of *Trichophyton mentagrophytes.* This same range was also found to increase the rate of appearance of mutations in the spores that survived. The maximum rate of mutation appearance among surviving spores was reached when 50 to 99% of the irradiated spores did not survive. These mutations differed from the parent strain in rate of growth, colony form, pigment production, and other characteristics. The mutations persisted through many transfers and could be called permanent alterations. Since sexual reproduction is not known in the dermatophytes, it was impossible for the authors to present positive proof of the genetical nature of these mutants, but by comparison, from experiments on *Neurospora* and other fungi, it seemed that the origin of the mutations in *Trichophyton* was definitely genetical. Some of the mutant strains were observed to produce much larger amounts of pigments than the controls. Comparative aspects of the problem of induction of mutations in fungi with ultraviolet irradiation are presented by HOLLAENDER, RAPER and COGHILL (1945*a*) on *Aspergillus niger* and by HOLLAENDER *et al.* (1945*b*) on *Neurospora crassa.* The techniques could usefully be applied in any investigation of the sort mentioned in the discussion on variant strains presented earlier in this chapter.

*Literature Cited:*—

1. BAKER, E. E. and C. E. SMITH. J. Infect. Dis. *70:* 51, 1942.
2. BEADLE, G. W. Physiol. Revs. *25:* 643, 1945.
3. BENHAM, R. W. Proc. Soc. Exptl. Biol. Med. *46:* 176, 1941.
4. BLANK, I. H. J. Invest. Dermatol. *2:* 67, 231, 1939.
5. BLOCK, R. J. and H. B. VICKERY. J. Biol. Chem. *93:* 113, 1931.
6. BODIN, E. Arch. de Parasit. *2:* 362, 1899.
7. BODIN. E. Arch. de Parasit. *5:* 5, 1902.
8. BONAR, L. and A. D. DREYER. Am. J. Pub. Health *22:* 909, 1932.
9. BONNER, J. T. Memo Rept. TSEAL 3-696-70H, Wright Field, Dayton, 1945.
10. BRENNER, W. Centrlblt. f. Bakt. II *40:* 555, 1914.
11. BURKHOLDER, P. R. and D. MOYER. Bull. Torrey Botan. Club *70:* 372, 1943.
12. CIFERRI, R. and P. REDAELLI. Atti dell'Ist. Botan. dell'U. di Pavia *6:* 55, 1935.
13. COHEN, H. R. Arch. Biochem. *4:* 145, 1944.
14. CONANT, N. F. Proc. Sixth Pacific Cong. *5:* 853, 1939.
15. CONANT, N. F. J. Bact. *41:* 563, 1941.
16. COUSINS, S. M. Studies in variation in the dermatophytes. Thesis, Harvard Univ., 1936.
17. DAVIDSON, A. M. and P. H. GREGORY. Canad. J. Res. *10:* 373, 1934.

18. Doudoroff, M. Enzymologia *5:* 239, 1938.
19. Emmons, C. W. and A. Hollaender. Am. J. Bot. *26:* 567, 1939.
20. Feeney, R. E., J. H. Mueller and P. A. Miller. J. Bact. *46:* 563, 1943.
21. Fleming, A. Lancet *ii:* 472, 1909.
22. Foster, J. W. Bot. Rev. *5:* 207, 1939.
23. Fries, N. Nature *152:* 105, 1943a.
24. Fries, N. Symbolae Botanicae Upsaliensis *7:* 73, 1943b.
25. Gage, S. de M. Am. J. Pub. Health *20:* 7, 1930.
26. Galloway, L. D. J. Textile Inst., Manchester *26:* T123, 1935.
27. Goddard, D. R. J. Infect. Dis. *54:* 149, 1934.
28. Goddard, D. R. and L. Michaelis. J. Biol. Chem. *106:* 605, 1934.
29. Goddard, D. R. and L. Michaelis. J. Biol. Chem. *112:* 361, 1935.
30. Gould, W. L. J. A. M. A. *96:* 1300, 1931.
31. Henrici, A. T. J. Bact. *39:* 113, 1940.
32. Hirsch, E. F. and H. Benson. J. Infect. Dis. *40:* 629, 1927.
33. Hoagland, D. R. Lectures on the Inorganic Nutrition of Plants. Chronica Botanica, Waltham, 1944.
34. Hollaender, A. and C. W. Emmons. J. Cell. Comp. Physiol. *13:* 391, 1939.
35. Hollaender, A., K. B. Raper and R. D. Coghill. Am. J. Bot. *32:* 160, 1945*a*.
36. Hollaender, A., E. R. Sansome, E. Zimmer and M. Demerec. Am. J. Bot. *32:* 226, 1945*b*.
37. Howell, A. Mycologia *33:* 103, 1941.
38. Jones, C. B. and D. K. Mecham. Arch. Biochem. *3:* 193, 1943.
39. Knight, B. C. J. G. Bacterial Nutrition. Med. Res. Council, Special Rept. Series, No. 210, London, 1936.
40. Knight, B. C. J. G. Vitamins and Hormones *3:* 105, 1945. *Ed.* R. S. Harris and K. V. Thimann, Academic Press, N. Y.
41. Landsteiner, K. The Specificity of Serological Reactions. Harvard University Press, Cambridge, 1945.
42. Leise, J. M. and L. H. James. J. Lab. Clin. Med. *30:* 119, 1945.
43. Leonian, L. H. and V. G. Lilly. J. Bact. *45:* 329, 1943.
44. Linderström-Lang, K. and F. Duspiva. Zeit. physiol. Chem. *237:* 131, 1935a.
45. Linderström-Lang, K. and F. Duspiva. Nature *135:* 1039, 1935b.
46. Luria, S. E. and M. Delbruck. Genetics *28:* 491, 1943.
47. Lwoff, A. Ann. Inst. Pasteur *61:* 580, 1938.
48. Macfayden, A. J. Path. and Bact. *3:* 177, 1894.
49. Mallinckrodt-Haupt, A. St. v. Zentrlbt. f. Bakt. I *103:* 73, 1927.
50. Mallinckrodt-Haupt, A. St. v. Zeit. f. Parasit. *5:* 217, 1933.
51. Mosher, W. A., D. H. Saunders, L. B. Kingery and R. J. Williams. Plant Physiol. *11:* 795, 1936.
52. Nannizzi, A. Ann. Mycologici *24:* 85, 1926.
53. Nickerson, W. J. Chronica Botanica *7:* 409, 1943.
54. Nickerson, W. J. and K. V. Thimann. Am. J. Bot. *30:* 94, 1943.
55. Nickerson, W. J., L. Irving and H. E. Mehmert. Arch. Dermatol. Syphilol. *52:* 365, 1945.
56. Nickerson, W. J. and J. B. Chadwick. Arch. Biochem. *10:* 81, 1946.
57. Oyama, T. Nayasaki Igakkwai Zasshi *15:* 2601, 1937. (Cited from Robbins and Kavanagh.)
58. Patterson, W. I., W. B. Geiger, L. R. Mizell and M. Harris. J. Res. Natl. Bur. Standards *27:* 89, 1941.
59. Richards, O. W. J. Biol. Chem. *96:* 405, 1932.
60. Robbins, W. J. and V. Kavanagh. Bot. Rev. *8:* 411, 1942.
61. Robbins, W. J. and R. Ma. Bull. Torrey Bot. Club *69:* 184, 1942.
62. Robbins, W. J., J. E. Mackinnon and R. Ma. Bull. Torrey Bot. Club. *69:* 509, 1942.
63. Robbins, W. J. and R. Ma. Bull. Torrey Bot. Club *70:* 190, 1943.
64. Robbins, W. J. and R. Ma. Am. J. Bot. *32:* 509, 1945.
65. Roberts, L. J. Path. and Bact. *3:* 300, 1894.
66. Roessler, W. G., E. J. Herbst, W. G. McCullough, R. C. Mills and C. R. Brewer. J. Bact. *51:* 572, 1946 (abstr.).
67. Rothman, S. Hygeia *23:* 436, 1945.

68. Rothman, S., A. M. Smiljanic and A. L. Shapiro. Proc. Soc. Exptl. Biol. Med. *60:* 394, 1945.
69. Routh, J. I. and H. B. Lewis. J. Biol. Chem. *124:* 727, 1938.
70. Schopfer, W. H. Plants and Vitamins. Chronica Botanica, Waltham, 1943.
71. Schopfer, W. H. and S. Blumer. Compt. rend. Soc. Phys. Hist. Nat. Geneve *59:* 106, 1942.
72. Ssadikow, W. S. Biochem. Zeit. *179:* 326, 1926.
73. Steinberg, R. A. Am. J. Bot. *6:* 330, 1919.
74. Steinberg, R. A. J. Agric. Res. *59:* 731, 1939a.
75. Steinberg, R. A. Bot. Rev. *5:* 327, 1939b.
76. Stewart, R. A. and K. F. Meyer. J. Infect. Dis. *63:* 196, 1938.
77. Tate, P. Biol. Revs. *4:* 41, 1929a.
78. Tate, P. Parasitology *21:* 31, 1929b.
79. Tatum, E. L. Ann. Rev. Biochem. *13:* 667, 1944.
80. van Niel, C. B. and A. L. Cohen. J. Cell. Comp. Physiol. *20:* 95, 1942.
81. Verujsky, Dm. Ann. Inst. Pasteur *1:* 369, 1887.
82. Waring, W. S. and C. H. Werkman. Arch. Biochem. *1:* 303, 1943.
83. Weidman, F. D. Arch. Dermatol. Syphilol. *15:* 415, 1927.
84. Williams, J. W. Proc. Soc. Exptl. Biol. Med. *31:* 586, 1934a.
85. Williams, J. W. Proc. Soc. Exptl. Biol. Med. *31:* 944, 1934b.
86. Williams, J. W. Proc. Soc. Exptl. Biol. Med. *32:* 624, 1935.
87. Williams, J. W. Science *83:* 396, 1936.
88. Williams, R. J. and J. M. Honn. Plant Physiol. *7:* 629, 1932.
89. Wirth, J. C. and F. F. Nord. Arch. Biochem. *2:* 463, 1943.



### *Chapter 10*

# METABOLIC PRODUCTS OF PATHOGENIC FUNGI

*by*

WALTER J. NICKERSON

**Introduction:**— Investigations on the metabolic products of pathogenic fungi are becoming more numerous. The possibilities of clinical use of polysaccharide preparations for desensitization of hypersensitive individuals are apparently stimulating efforts in this direction. Pigment production, in a few instances, seems to have value as a taxonomic aid for species identification. The chapters on metabolism in this volume deal in part with metabolic products but the discussion here will concern itself with pigments, polysaccharides and proteins.

**Pigment Production:**— Studies on the production of pigment by members of the pathogens are frequently complicated, as with the dermatophytes, by the occurrence of pleomorphism. Pleomorphic colonies (*sensu* SABOURAUD) have lost their ability to produce pigments. In the genus *Trichophyton* the production of a rose-brown pigment on many media is usually characteristic of *T. mentagrophytes* (*gypseum*), while strains of *T. rubrum* produce a deep wine-red color on many media. BENHAM (1943) mentioned observing several strains typical of *T. mentagrophytes* (*gypseum*) in every way except that they produced the wine-red color on dextrose agar media.

*Pigments of the dermatophytes.* — Many of the dermatophytes produce pigments, especially when grown on media rich in carbohydrate. At 30° C. on a glucose or maltose medium, *Trichophyton Megnini* (*gallinae*) produces a bright red pigment which diffuses into the medium; *T. violaceum* also produces a reddish diffusible pigment. *T. mentagrophytes* (*gypseum*) and *T. tonsurans* (*crateriforme*) often develop yellowish or reddish brown pigments, especially in old cultures that are drying up. Species of *Microsporum* as *M. canis* often form yellow or brown pigments. SABOURAUD (1910) stated that the pigments of the dermatophytes are acids and precipitable by alkalis. TATE (1929*a*) examined the pigments produced by species of *Trichophyton;* the pigments of several species (*T. Megnini, T. Sabouraudi, T. rubrum,* and *T. mentagrophytes*) were found to be red to reddish brown, easily soluble in dilute acids and acid alcohol, but only very slightly soluble in dilute alkali. On extraction the color in acid solution was yellow changing to red or reddish brown in alkaline solution, the color change being easily reversible and the pigment thus as indicator of hydrogen ion concentration. The pigments are not destroyed by boiling; they can be extracted by ether and chloroform from aqueous acid solutions. In alkaline solution the pigment can be reduced to a pale yellow with sodium hydrosulfite and reoxidized to a red color in contact with air; the oxidation-reduction is reversible. Tate suggested the pigments might be of an anthracene nature, resembling the yellow pigments of the lichen *Physcia.*

Thompson (1943) confirmed Tate's findings and pointed out that the pigments of *T. rubrum* and *T. mentagrophytes* are soluble in acetone; in solution, oxygen prompts a color change of the pigment to a deep wine-red so that the two colors become indistinguishable. On dilution of the acetone solution with water a partial precipitation occurs; the pigment remaining in solution changes to a cherry color which deepens at *p*H 8 on adding alkali, becoming a blue-lavender above *p*H 9. On standing in alkaline solution the color was found to turn yellow but the lavender color might be regenerated by oxygen, though not by nitrogen, hydrogen sulfide, or carbon dioxide. This pigment seems to be a *p*H indicator with an oxidized and a reduced form. Some comparisons of the pigments of dermatophytes are presented in Table 1. This field merits investigation of the chemical nature of these colored compounds, and the possibility that the pigments serve for oxygen transport in these fungi deserves study. It would appear from Table 1 that it is possible there may be only one pigment, *i.e.* one chemical substance, responsible for the coloration developed in cultures of dermatophytes. The *p*H and oxidation-reduction potential of the culture would determine the actual color developed. In some unpublished work Jillson and Nickerson found that a yellow color developed in two-member pure cultures of *Trichophyton rubrum* and *Candida albicans* on corn meal agar (culture acid), while

Table 1.—*Pigments of Dermatophytes:*—

| Organism | *Color in culture* | *Color in acid solution* | *Color in alkaline solution* | *Oxidized color* | *Reduced color* | *Reference* |
|---|---|---|---|---|---|---|
| *M. canis* ........ | yellow or brown | ...... | ...... | ...... | ...... | Tate (1929*a*) |
| *T. mentagrophytes*<br>*T. Megnini* .....<br>*T. rubrum* ......<br>*T. Sabouraudi* ... | red to reddish brown | yellow | red to reddish brown | red | pale yellow | Tate |
| *T. mentagrophytes*<br>*T. rubrum* ...... | red to reddish brown | ...... | ...... | red | ...... | Thompson (1943) |
| *T violaceum* .... | red | ...... | ...... | ...... | ...... | Tate |
| *T. tonsurans* .... | yellowish to reddish brown | ...... | ...... | ...... | ...... | Tate |

the same strain of *T. rubrum* alone on corn meal agar (culture alkaline) developed a red color.

*Green pigment of Candida albicans.* — A faint greenish color produced by colonies of some cultures of *Candida albicans* was noted by ASHFORD (1917). MARTIN, JONES, YAO and LEE (1937) and MARTIN and JONES (1940) described six species of *Candida,* of which it was noted that *C. stellatoidea* produced considerable amounts of a green pigment. This pigment was investigated by JONES and PECK (1940) with the thought that it might prove an additional character by which to distinguish *C. albicans* from *C. stellatoidea* (easily separable, however, on biological differences and pathogenic action). On blood agar plates, with Sabouraud's broth as the diluent, a greenish zone was easily discernible around colonies of *Candida stellatoidea;* in these cultures the pigment resembled methemoglobin. Plates containing 25 to 50 colonies were suitable to demonstrate pigment production; too heavy seeding prevented individual colonies from reaching a large size and no pigment could be noticed in such plates with minute colonies.

JONES and PECK found that a thin greyish-green layer persisted on the surface of the masses of cells of *C. stellatoidea* that had been centrifuged from saline suspensions. The pigment was readily soluble in acetone, yielding a green solution. To obtain sufficient material for chemical investigation, fresh cells were extracted with acetone. *C. stellatoidea* produced ten times the quantity of pigment produced by *C. albicans.* The pigment proved to be *p*H indicator with a blue-green color in acid solution and yellow in alkaline solutions. The substance appeared to be a weak acid, unstable in nature.

It seems fairly certain that the pigment studied by JONES and PECK was not identical with riboflavin, from the data they reported on the light absorption characteristics of an ethyl acetate solution of the crude green pigment isolated from *C. stellatoidea.* Using a spectrophotometer they observed an absorption maximum at 6550 Å. The absorption maxima for riboflavin (shown by HOLLIDAY and STERN, 1934, to be due to the alloxazine nucleus) were found by KUHN *et al.* (1933) for nearly neutral aqueous solutions to be pronounced at 2200 and 2650 Å and of secondary magnitude at 3650 and 4450 Å.

*Synthesis of riboflavin by species of Candida.* — Production of considerable amounts of riboflavin by species of *Candida* has recently been reported by TANNER, VOJNOVICH and VAN LANEN (1945). An interesting result of their study was the influence shown for iron on the amount of riboflavin secreted into the medium. As shown in Table 2, the concentration of iron for maximum secretion of riboflavin is in the range of 0.5 — 1.0 $\mu$g. iron per 100 ml. of medium. This is about 1/100th the critical concentration of iron for the production of riboflavin by the anaerobic bacterium *Clostridium acetobutylicum* shown by SAUNDERS and MCCLUNG (1943). The optimum iron concentration for growth of the species of *Candida* shown in Table 2 is much higher (*ca.* 50 µg./100 ml.) than the optimum level for riboflavin secretion. This indicates clearly that riboflavin production is enhanced by a deficiency of iron for growth. The impression is gained that under these conditions of poor nutrition, the release of riboflavin into the medium may actually be a pathological phenomenon.

TABLE 2.—*Production of riboflavin by different species of yeasts:*—

| ORGANISM | *µg Fe/ 100 ml medium* | *Dry wt. of yeast gm/100 ml med.* | RIBOFLAVIN *produced µg/ml* | *Reference* |
|---|---|---|---|---|
| *Candida* | 0.0 | 0.27 | 108 | |
| *Guilliermondi* | 0.5 | 0.57 | 123 | TANNER *et al.* |
| (NRRL 488) | 1.0 | 0.66 | 128 | (1945) |
| | 10.0 | 0.89 | 7.2 | |
| | 50.0 | 0.92 | 3.2 | |
| *Candida* | 0.0 | 0.21 | 107 | |
| *Guilliermondi* | 0.5 | 0.67 | 125 | TANNER |
| (NRRL 324) | 1.0 | 0.61 | 157 | |
| | 10.0 | 0.89 | 16.5 | |
| | 50.0 | 0.82 | 10.6 | |
| *Candida* | 0.0 | 0.42 | 195 | |
| *flareri* | 0.5 | 0.49 | 216 | TANNER |
| (NRRL 245) | 1.0 | 0.55 | 216 | |
| | 10.0 | 1.12 | 8.9 | |
| | 50.0 | 1.31 | 4.1 | |
| *Candida* | | | | ROGOSA |
| *pseudotropicalis* | ... | ... | 0.06 | (1943) |
| *Torulopsis* | 0.0 | 0.116 | 0.14 | |
| *utilis* | 10.0 | 0.420 | 0.32 | LEWIS |
| (NRRL 900) | 30.0 | 0.483 | 0.44 | (1944) |
| | 100.0 | 0.476 | 0.46 | |
| | 300.0 | 0.604 | 0.61 | |
| *Ashbya* 4 days | ... | ... | 70 | WICKERHAM |
| *gossypii* | | | | *et al.* (1946) |
| (NRRL 1056) 6 days | ... | ... | 217 | |
| 8 days | ... | ... | 381 | |

This impression is supported by the results of WICKERHAM, FLICKINGER and JOHNSTON (1946, *see* Table 2) on *Ashbya gossypii* which produced riboflavin in quantity, apparently independently of the iron concentration, but showed increasing production with the age of the culture. The cultures were grown for 8 days with aeration in a medium containing initially 2% of a commercial dextrose (Cerelose); after 4 days only 0.03% dextrose remained in the medium and for the remaining 4 days the yeast cells were subjected to what was tantamount to aeration in a non-nutrient medium—a process which STIER and MACINTYRE (1943) showed was accompanied by considerable loss of riboflavin from the cells of *Saccharomyces cerevisiae* into the suspending medium. There is considerable evidence that the loss of growth factors (excepting *p*-amino benzoic acid) into a non-nutrient buffer does not occur in the absence of oxygen (STIER and MACINTYRE, 1942; NICKERSON and CARROLL, 1943; LEWIS, STUBBS and NOBLE, 1944).

*Black pigment of Sporotrichum.* — The black pigment, found exclusively in the spores, is produced by most cultures of *Sporotrichum.* From the first description of this organism by SCHENCK (1898) nearly every worker dealing with the group has remarked on the blackish pigment developed by these organisms in cultures. Growth is at first white and the rate of formation of the black pigment is subject to considerable variation with strain differences, composition of the medium, oxygen supply, etc. Microscopically the pigment is seen confined to the spores; mycelia are usually colorless. The black pigment may be formed evenly over the surface of a colony or its distribution may be limited to small areas, most of the colony remaining white; in the latter case, spores in the non-pigmented areas are white. DAVIS (1915) isolated single spores from pigmented and non-pigmented areas and obtained cultures that were evenly black or evenly white; he did not report any sectoring occurring in ten subcultures following isolation of a spore. Both black and white types appeared to be equally pathogenic when inoculated into animals. DAVIS made a brief study of the black pigment and found it could not be extracted from the spores by water, dilute acids, dilute alkali, hot alcohol, ether, xylol, or chloroform. Strong acids dispersed mycelia and spores and destroyed the pigment. No test for iron[1] could be obtained with the pigment in the spores by staining. Cultures normally producing the pigment were unable to do so under low oxygen tension. DAVIS, however, did not come to any conclusion as to the nature of the black pigment.

Recent experiments by NICKERSON (previously unpublished) indicate that the black pigment of *Sporotrichum Schenckii* is probably a melanin. The presence of tyrosinase could be demonstrated in spore masses but not in mycelia free of spores. A substrate oxidizable by tyrosinase prepared from mushrooms (*Coprinus micaceus*) could be demonstrated in both spore masses and in mycelial masses devoid of spores. No study was made of white-spored strains, since none was at hand. The addition under aseptic conditions of small amounts of spore masses, as nearly as possible teased free of mycelia then filtered with suction through glass wool, to a sterile solution of tyrosine (0.033 g./100 ml. water) resulted in darkening of the solution. The reaction was not rapid and darkening progressed slowly through the hallochrome stage for several weeks. Grinding spore masses with chloroform before addition to a tyrosine solution caused the darkening to go relatively faster. Similar addition of mycelial masses practically free from spores did not cause darkening of the solution. With a preparation of tyrosinase from the mica ink cap mushroom (*Coprinus micaceus*) there was a marked increase in oxygen uptake on adding extracts of either mycelia or spores of *Sporotrichum Schenckii.* Figure 1 is illustrative of an experiment wherein oxygen uptake was measured in the Scholander volumetric respirometer, using the enzyme preparation and an aqueous solution extracted from fungus mycelia ground with chloroform.

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[1] LINOSSIER (1891) isolated a black alkali-soluble hematin pigment from the spores of *Aspergillus niger;* the pigment contained iron and exhibited a resemblance to hemoglobin. Deficiencies of iron, manganese, copper, and oxygen are known to interfere with the formation of the pigment and spores; leucospores are rarely found (*see* FOSTER, 1939).

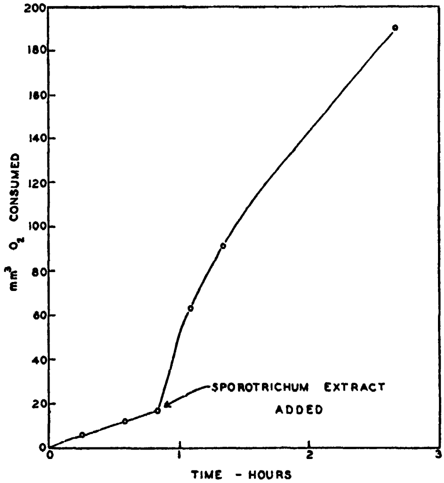

**Figure 1.** — Increase in oxygen consumption obtained on adding an aqueous extract of mycelia of *Sporotrichum Schenckii* to a tyrosinase preparation (acetone precipitate from minced *Coprinus micaceus* fruiting bodies).

Apparently the black pigment of *Sporotrichum Schenckii* is a melanin produced by the enzymatic oxidation of some substrate by tyrosinase. Evidently the enzyme is confined to the spores, though an oxidizable substrate may be extracted from the mycelia as well as from the spores. The findings of Davis (1915) are compatible with the present finding that the black pigment is a melanin.

Melanin production is quite widespread; the pigment is found in hair, insects, fleshy fungi, cut surfaces of apples and potatoes, etc.; Wigglesworth (1938) gives an account of its production in insects. Oxygen is required for the formation of melanin since the pigment is formed by the enzymatic oxidation of a colorless precursor, aromatic in structure, termed a "chromogen." In insects the melanin-producing enzymes are generally diffused throughout the animal and it is the localization of chromogens that determines the color-patterns developed. There is a considerable variety of chromogens, or substrates, upon which the melanogenic enzymes may act; tyrosine (monohydroxyphenylalanine), "dopa" (3, 4-dihydroxyphenylalanine), other polyphenols, dioxyphenylacetic acid, etc.

There is a second pigment, usually less conspicuous, produced in cultures of *Sporotrichum.* This is a yellow to yellow-brown diffusible color. Cultures on agar come to have a yellow-brown color with time, evidently caused by diffusion of this pigment into the agar.

**Polysaccharides:**— At least in the case of diseases brought about by the pathogenic yeast-like fungi, the polysaccharides produced seem to occupy an important position. In the case of animals that have been immunized with whole cells of *Candida albicans,* circulating antibodies are developed which are capable of precipitating polysaccharides isolated from the cells of *C. albicans* (*see* KESTEN *et al.,* 1930, and NEGRONI, 1936). Hypersensitivity to polysaccharides may, however, develop in some cases when the presence of such precipitins cannot be shown. PECK, MARTIN and HAUSER (1940) pointed out that such hypersensitivity is probably of importance in certain cases of chronic infection, especially is some cases of American blastomycosis (Gilchrists's disease).

*Polysaccharides of Blastomyces dermatitidis.* — PECK, MARTIN and HAUSER undertook a study of the preparation, properties, and biologic activities of some polysaccharides obtainable from *Blastomyces dermatitidis,* the causative organism of American blastomycosis. At least two different polysaccharides (one of which seemed to yield mannose after acid hydrolysis) were isolated. Cells were suspended in a saline solution, containing 1% phenol, for 4 days, then removed by centrifugation; polysaccharides were isolated from the supernatant liquid by treatments with sodium acetate and ethanol. The crude polysaccharide preparations obtained in yields of about 0.01 gram per gram (dry weight) of cells were faintly buff-colored hygroscopic powders. Very dilute solutions of the purified polysaccharides fixed complement with sera prepared by immunizing rabbits with ground cells of *Blastomyces dermatitidis.* Clinically, patients with American blastomycosis in the allergic stage of the disease responded to intracutaneous injection of the polysaccharide with a swelling that reached maximum size within 24 hours. With one patient intravenous injection of the polysaccharide decreased carbohydrate sensitivity and abolished the sterile abscess-formation produced in an allergic patient by injection of heat-killed whole organisms.

*Immunologically active polysaccharide from Coccidioides immitis.* — Coccidioidin prepared from filtrates of old cultures of *C. immitis* is used as a diagnostic aid with coccidioidomycosis; a skin reaction is obtained on intra-dermal injection into sensitive persons. HIRSCH and D'ANDREA (1927) precipitated with alcohol from filtrates of old cultures of *C. immitis* a substance yielding 20-40% reducing sugars on hydrolysis; glucose was identified in the hydrolyzate. A more extensive investigation of the polysaccharide produced by this organism was undertaken by HASSID, BAKER and MCCREADY (1943). Concentrating the filtrate from a 2-months'-old culture by the use of collodion ultrafilters, through which the skin reaction substance would not pass, these authors on analysis found the polysaccharide to consist of units of galacturonic acid, glucose, and some unidentified sugar in the approximate ratio of 1:6:3 respectively. A nitrogenous compound, apparently other than protein, was found to be associated with the polysaccharide. Clinically the purified polysaccharide gave a skin reaction with sensitive individuals and a positive precipitative reaction. An acetyl derivative of the original polysaccharide, however, yielded a polysaccharide which still produced a positive precipitative reaction but no longer elicited a skin reaction.

*Capsule of Torulopsis neoformans.* — In the course of a study on the growth requirements of different yeasts, ASCHNER, MAGER and LEIBOWITZ (1945) found that *Torulopsis rotundata* and *T. neoformans* gave a steel-blue color reaction with iodine. Cells of these yeasts had been grown in a simple inorganic salt medium with glucose, ammonium sulfate, and thiamine. The substance responsible for the color reaction was found in the medium in which the cells had grown as well as in the large capsules with which cells of these yeasts are surrounded, but it could not be detected within the cells proper. Several grams of the color-responsible substance was prepared by precipitating it from cell-free media with alcohol; the substance came down as white, thread-like masses. The precipitated material was slightly soluble in the cold water, giving an opalescent solution. It was found that the material was of a carbohydrate nature and contained at least two fractions, one of which was a pentosan, the other being an amylose. Spectrophotometric analysis of the iodine complex showed it to have an extinction curve identical with an iodine complex preparation from pure potato amylose. Through the action of amylases of plant and animal origin, complete disappearance of the iodine color reaction could be caused; a fraction remaining after this enzymatic treatment could be precipitated with alcohol, the precipitate giving a strongly positive test for pentoses.

Many carbon sources were examined for their suitability as substrates; it was found that any compound supporting growth of these yeasts was also suitable for the production of the amylose fraction. Glucose-l-phosphate, inulin, starch, and glycogen were found not to support growth. With several nitrogen sources examined it was found necessary to keep the *p*H at 5 (or even more acid) to permit amylose formation, though pentosan production could take place in the alkaline ranges.

Of twenty-five other yeasts examined, including uncapsulated species of *Torulopsis,* only *T. rotundata* and *T. neoformans* produced amylose. The authors mentioned are investigating the serological properties of the external polysaccharides. BENHAM (1935) has shown that rabbits produced antibodies following the injection of decapsulated cells of *T. neoformans* (capsule removed with acid) but did not do so following the injection of whole cells.

*Miscellaneous polysaccharides.* — It might be the case that the skin lesions produced by injection of spores of *Coniosporium corticole* as reported by TOWEY, SWEANY and HURON (1932) in their study of coniosporiosis are actually prompted by a polysaccharide (or by a melanin) type substance. These authors found no appreciable skin reaction on injection of the fat soluble and protein fraction from the spores, but the black residues from the above preparations produced serious lesions similar to those produced on injection of the spores.

**Proteins:**— There is some information available on the enzymes of the dermatophytes from the work of TATE (1929*a, b*) and a little information from scattered sources on immunologically active proteins of various genera. There is a scarcity of information on structural and storage proteins of all the pathogens.

*Enzymes of the dermatophytes.* —Using acetone powders prepared (according to ONSLOW, 1923) from young cultures of representative species TATE (1929*b*) made a general survey of the enzymes of the dermatophytes. With the method employed there is obtained from the mycelium a fine, dry powder containing most of the enzymes uninjured, which keeps for weeks without deteriorating. Cultures of *Microsporum canis* (*lanosum*), *M. Audouini, Trichophyton tonsurans, T. Schoenleinii,* and *T. mentagrophytes* (*radiolatum*) were used; normal and pleomorphic strains of *T. mentagrophytes* were studied comparatively. A proteolytic enzyme active in alkaline media was present in all species; this trypsin-like enzyme hydrolyzed intact proteins (casein) producing free amino acids. No proteolytic enzyme active in acid media (pepsin) was found in any strain. The amount of proteolytic enzyme present was found to vary with the species; *T. mentagrophytes* was comparatively high in its content of this enzyme. The normal strain of *T. mentagrophytes* showed a greater proteolytic activity than did the pleomorphic form; the latter strain was comparatively rich in urease and amygdalase, both poorly represented in the normal strain.

### *Literature Cited:* —

ASCHINER, M., J. MAGER and J. LEIBOWITZ. Nature *156:* 295, 1945.
ASHFORD, B. K. Am. J. Med. Sci. *154:* 157, 1917.
BENHAM, R. W. J. Infect. Dis. *57:* 255, 1935.

Benham, R. W. Rept. Conf. on Dermatophytes, Div. Med. Sciences, Nat. Res. Council, Nov. 8, 1943.
Davis, D. J. J. Infect. Dis. *17:* 174, 1915.
Foster, J. W. Bot. Rev. *5:* 207, 1939.
Hassid, W. Z., E. E. Baker and R. M. McCready. J. Biol. Chem. *149:* 303, 1943.
Hirsch, E. F. and D. d'Andrea. J. Infect. Dis. *40:* 629, 634, 638, 1927.
Holliday, E. R. and K. Stern. Ber. deutsch. chem. Ges. *67:* 1352, 1934.
Jones, C. P. and R. L. Peck. J. Bact. *39:* 605, 1940.
Kesten, H. D., D. H. Cook, E. Mott and J. W. Jobling. J. Exptl. Med. *52:* 813, 1930.
Kuhn, R., P. György and T. Wagner-Jauregg. Ber. deutsch. chem. Ges. *66:* 1034, 1933.
Lewis, J. C. Arch. Biochem. *4:* 217, 1944.
Lewis, J. C., J. J. Stubbs and W. M. Noble. Arch. Biochem. *4:* 389, 1944.
Linossier, G. Compt. rend. Acad. Sci. Paris *112:* 489, 1891.
Martin, D. S., C. P. Jones, K. F. Yao and L. E. Lee. J. Bact. *34:* 99, 1937.
Martin, D. S. and C. P. Jones. J. Bact. *39:* 609, 1940.
Negroni, P. Ann. Parasitol. *14:* 511, 1936.
Nickerson, W. J. and W. R. Carroll. J. Cell. Comp. Physiol. *22:* 21, 1943.
Onslow, M. W. Practical Plant Biochemistry. 2nd ed., Univ. Press. Cambridge, 1923.
Peck, R. L., D. S. Martin and C. R. Hauser. J. Immunol. *38:* 449, 1940.
Robbins, W. J. and R. Ma. Am. J. Bot. *32:* 509, 1945.
Rogosa, M. J. Bact. *45:* 459, 1943.
Sabouraud, R. Les Teignes. Masson et Cie, Paris, 1945.
Saunders, A. and L. S. McClung. J. Bact. *46:* 575, 1943.
Schenck, R. B. Bull. Johns Hopkins Hosp. *9:* 286, 1898.
Stier, T. J. B. and C. F. MacIntyre. J. Cell. Comp. Physiol. *20:* 385, 1942.
Tanner, F. W., Jr., C. Vojnovich and J. M. van Lanen. Science *101:* 180, 1945.
Tate, P. Biol. Revs. *4:* 41, 1929a.
Tate, P. Parasitology *21:* 31, 1929b.
Thompson, K. W. Rept. Conf. on Dermatophytes, Div. Med. Sciences, Nat. Res. Council, Nov. 8, 1943.
Towey, J. W., H. C. Sweany and W. H. Huron. J. A. M. A. *99:* 453, 1932.
Wickerham, L. J., M. H. Flickinger and R. M. Johnston. Arch. Biochem. *9:* 95, 1946.
Wigglesworth, V. B. Insect Physiology. Methuen, London, 1938.

## Chapter 11

# THE LIPIDS OF FUNGI WITH SPECIAL REFERENCE TO PATHOGENIC FUNGI

*by*

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**Introduction:**— The chemistry of microorganisms is attracting increased interest. Investigations dealing with the isolation, purification and characterization of cell-constituents and metabolic products of fungi and bacteria have long been yielding data of microbiological significance. Many of the results obtained in these studies have also been of the utmost value in facilitating the better understanding and therapy of infectious diseases. The antibiotic substance, penicillin, may be cited as an example of a metabolic product of a fungus which has achieved a very important clinical application as a therapeutic agent (HERRELL, 1945). Studies carried out on the specific polysaccharides of pneumococci have resulted in the recognition of new phases of immunology, and have aided in the development of the therapy of pneumonia (HEIDELBERGER, 1927, 1939). Investigations of the lipids of human tubercle bacilli (ANDERSON, 1939; SABIN, 1932) have led to the isolation of unusual types of lipid constituents which possess the property of stimulating the formation of tuberculous tissue when injected into animals. The latter studies have provided a means for relating lesions of tuberculosis to specific components of tubercle bacilli.

The lipids of pathogenic fungi represent a very important fraction of the cellular protoplasm. Investigations dealing with the chemistry of this interesting group of substances have been, however, rather limited. The present survey is therefore mainly concerned with data obtained on two pathogenic, yeast-like fungi, *Blastomyces dermatitidis* (PECK and HAUSER, 1938, 1940) and *Candida albicans* (PECK and HAUSER, 1939, 1940) which are representative of a fairly considerable group of pathogenic organisms. Sufficient data on the lipids of non-pathogenic fungi, and pathogenic and non-pathogenic bacteria are available to permit comparison of the various types of compounds isolated.

The role of the lipid constituents of the cells of pathogenic fungi and bacteria in infectious disease mechanisms is undoubtedly significant. The lipids of pathogenic microorganisms are capable of interfering with enzyme systems as shown by JOBLING and PETERSEN (1914) and PECK (1942). Caseation phenomena in tuberculosis, for example, may be attributed in part to such interference with a normal

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host mechanism. Preliminary studies of the effects of tubercle bacillus lipids on animal enzyme systems, both *in vitro* and in the living animal body by GERSTL and TENNANT (1941, 1942, and 1943) and by GERSTL, TENNANT and PELZMAN (1945) have given further insight into this type of reaction. Inhibition appears to depend on the molecular weight, structure, and configuration of the lipid, as well as on such physico-chemical factors as surface activity and adsorption. The quantitative study of the inhibition of crystalline trypsin by soaps of the fatty acids from *Blastomyces dermatitidis* and from tubercle bacilli, and by soaps of a number of other pure fatty acids by PECK (1942) brings out more clearly the effect of molecular weight of the fatty acid on the degree of inhibition. These studies suggest possibilities for further experimental work on the effects of lipids on tissue enzymes using both *in vitro* and *in vivo* systems. The results should aid in the interpretation of certain disease mechanisms.

In the case of tuberculosis, of all the substances thus far isolated from human tubercle bacilli the lipids have shown by far the most damaging effect upon the cells of the host (SABIN, 1932). The formation of lesions closely resembling those produced by the living organisms has been accomplished in rabbits and guinea pigs by the injection of the tubercle bacillus phospholipid, and in mice by means of the wax fraction (THOMAS and DESSAU, 1939). The phospholipid fraction did not appear to elicit in mice the degree of response noted in rabbits; this illustrates an interesting difference in specific susceptibility. Similar studies carried out in mice with the lipids of *Blastomyces dermatitidis* by BAKER (1942) showed that the phospholipid obtained from this pathogenic fungus exerted a marked effect on cellular proliferation. It nevertheless could not alone be responsible for the necrotic reactions observed on injection of the living or killed organisms.

The heterophile antigens of Forssman, lipid-protein complexes sometimes containing carbohydrates, are immunologically active substances in which the lipid fractions act as haptenes (ZINSSER and BAYNE-JONES, 1939, p. 46). In the capacity of haptenes, lipids of pathogenic microorganisms may influence the immune and (in the case of fungi, particularly) allergic responses of the host. More data are needed in order to provide a basis for the thorough evaluation of the degree of participation of lipids of pathogenic microorganisms in the pathological reactions accompanying the diseases. The necessary investigations will require carefully purified lipid preparations from a variety of pathogenic forms, as well as information concerning the chemical structure of these lipids. Joint chemical, microbiological, immunochemical, and pathological studies are much needed.

The capacity of pathogenic microorganisms to survive in the tissues of the host depends in part on the lipid system of the invading cells. Reasoning from the findings on red cell stromata by PARPART and DZIEMIAN (1940), it may be conceived that the cell membranes of the invading pathogenic microorganisms contain a large amount of lipid material, possibly composed of phospholipids and sterols. The permeability of the invading cells, insofar as this is determined by the lipid components of the membrane, will be a function of the ratio of the hydrophilic colloid (phospholipid) to the hydrophobic colloid (sterol). The nature of the substances capable of penetrating the cell membrane will be determined to a considerable degree by their lipid-solubility. Penetration will also be a function of the relative amounts of acidic and basic cell components (lipid or otherwise) held in the membrane.

From the pharmacological viewpoint, the activity of certain therapeutic agents, particularly antibiotic substances, must be in part dependent on their ability to penetrate into the cells of pathogenic microorganisms in the host tissues. It becomes important to know more about the character of the cell membrane, the factors which influence its permeability to acidic or basic antibiotics of suitable lipid-solubility, and the nature of the lipids in the membrane. Studies carried out on synthetic monolayers give some insight into this problem (*see* HÖBER, 1945). Physical and chemical studies of the lipid composition of pathogenic microorganisms may permit deductions as to types of antibiotic substances most likely to be specific for various pathogenic organisms.

It is greatly to the interest of a better understanding of infectious disease mechanisms to provide as much information as possible on the composition of the cells of pathogenic microorganisms. The isolation and characterization of the components of microorganisms is in itself an arduous and complicated task. In many instances specialized technique is required. For the best evaluation of the results of these studies it is important that the greatest care be taken to avoid the occurrence of chemical changes in the lipid fractions during isolation and before physico-chemical measurements are made and before pathological or immunological testing.

**General Methods of Extraction and Separation of Lipid Fractions:**— The lipids of fungi and bacteria have been studied by gross chemical methods and by such microchemical procedures as the examination of cells stained with lipid-specific dyes. The present discussion concerns only the results obtained by the former methods. In order to obtain the most significant results from chemical studies of the lipids of microorganisms, it is highly desirable to obtain the various fractions in a condition as similar as possible to that in which they exist in the living cells. This is also of importance whenever biological studies of the lipid fractions are to be carried out. It is therefore necessary to use the mildest methods in the isolation and purification of the various fractions. For this purpose, only purified neutral solvents, preferably redistilled, should be employed. In order to prevent oxidation of unsaturated lipids, air should be displaced from all solvents and containers with carbon dioxide or nitrogen. Extractions should be carried out at room temperature; concentration of extracts should be carried out under reduced pressure in an atmosphere of carbon dioxide or nitrogen. The methods recently developed by ANDERSON and co-workers (1, 2, 3, 4, 12, and 50) during studies of the lipids of tubercle bacilli are particularly recommended for this type of work.

Qualitatively, the composition of the lipids of microorganisms appears to be specific for the organism studied. Quantitative composition, however, depends on a number of variable factors among which are the composition of culture media, the temperature and other physical growth conditions, the methods of treatment of cells during harvesting and prior to extraction, and the extraction procedures. Failure to consider these factors, as well as the lack of sufficient material to enable satisfactory separation of fractions, has deprived much of the older quantitative data (CZAPEK, 1922; ZELLNER, 1907) of significance. The most suitable medium for growth of microorganisms for isolation and characterization of constituents is, of course, a synthetic medium of uniform composition containing only simple organic compounds of known constitution together with inorganic salts. Using such a medium, one can be certain that the complex constituents of the cells such as lipids, carbohydrates, and proteins have all been synthesized from very simple known compounds. It is frequently the case, however, that virulent pathogenic fungi will not grow on such a simple medium, and more complex organic nutrients must be added to induce satisfactory growth. In such cases, especial care must be taken to prevent the medium from accompanying the cells during harvesting. Also, the interpretation of results obtained with organisms grown on the more complex media necessitates more caution. The cells should be washed free of adhering medium with water or an isotonic buffer solution during harvesting, then introduced directly into the extraction solvents.

The freshly harvested cells of the organism to be investigated are suspended in alcohol-ether (1:1) and allowed to stand at room temperature for several days. The alcohol-ether extract is then removed and replaced with fresh solvent. Two or three such extractions may be followed by one or more extractions with chloroform. The combined alcohol-ether extracts are concentrated under reduced pressure to an aqueous suspension of lipid material and the lipids are extracted into ether. The ethereal solution, after drying over anhydrous sodium sulfate or magnesium sulfate, is concentrated to small volume and treated with acetone, whereupon the phospholipids separate as an amorphous insoluble fraction. The phospholipids may be purified somewhat by repeated precipitation with acetone from ether. The acetone mother liquors on removal of solvent yield the so-called acetone-soluble lipid fraction. The chloroform extracts on removal of solvent under reduced pressure yield a lipid fraction which in the case of fungi generally resembles the acetone-soluble lipids and may be conveniently combined with this fraction. In the case of the acid-fast bacteria (ANDERSON *et al.*, 1937; ANDERSON, 1939), and of diphtheria bacteria (CHARGAFF, 1933), however, the chloroform extracts yield considerable amounts of wax-like fractions which differ markedly from the acetone-soluble lipids. A wax-like fraction may sometimes be separated by cooling an acetone solution of the acetone-soluble lipids, whereupon the wax fraction usually separates out as a solid which may be collected by centrifugation or filtered off and dried. It is desirable to conduct a preliminary examination of the properties of the chloroform extract before combining it with the acetone-soluble fraction.

The procedures described above represent a general method for removal of the readily extracted lipids from the cells of microorganisms. Many variations of these procedures have, of course, been employed. Insofar as possible, it is essential to use living cells for the first extractions rather than cells which have been killed, since most preliminary treatments cause more or less extensive hydrolytic and oxidative modifications of certain of the lipid fractions, particularly of the phospholipids (see below).

After removal of lipids by means of alcohol-ether and chloroform, there still remain in the cells of bacteria and fungi considerable amounts of lipids which cannot be removed by exhaustive extraction with neutral solvents. These lipids appear to be held in part by chemical linkages to carbohydrate and/or protein elements of the cell structure. Some mechanical occlusion also occurs in the larger or smaller cell aggregates since the cells are seldom in completely uni-cellular suspension (TURPEINEN, 1936) during extraction. These lipid fractions have been referred to by ANDERSON (3, 4) as firmly bound lipids. A mild method for liberating such firmly bound lipids was developed for use on human tubercle bacilli by ANDERSON and coworkers (4, 12, and 50). After extraction with neutral solvents, the cells were treated with 25% alcohol which removed considerable amounts of polysaccharides, pigments, non-protein nitrogen constituents, etc. The cells were then digested with alcohol-ether (1:1) containing 1% hydrochloric acid at a temperature of 50° C. for about five hours. The cell residues were filtered off, washed with alcohol and ether and afterwards exhaustively extracted with ether and chloroform. The extracts were worked up in the usual way. Variations of this procedure have also been described by PECK and HAUSER (1940). These methods appear to be best suited to isolation of the firmly bound lipids in relatively unchanged form for chemical study. The use of alkaline hydrolysis in liberation of bound lipids possesses the inherent drawback that the firmly bound lipids are largely saponified. From such material little chemical data of structural value can be obtained. The use of stronger acid is likewise to be recommended only where extensive hydrolysis of the lipid material does not matter.

An acid disintegration method has been worked out by TURPEINEN (1935, 1936) for estimation of the total lipids present in fungi. In this method the cells are digested in 5 $N$ hydrochloric acid on the steambath for twenty minutes, cooled, and extracted with ether. The ether solution is taken to dryness and the residue is dissolved in petroleum ether. The weight of the petroleum ether-soluble lipids dried at 50° C. is a measure of the total lipids. The values found in this way will be slightly low due to partial hydrolysis, but the data presented (TURPEINEN, 1936) indicate that *hydrolysis* of the lipids is by no means complete. The data reported would indicate that the milder methods of extraction of lipids leave a rather significant amount of unextracted lipids in the cell residues.

The methods employed in further purification and in the characterization of individual lipid fractions will not be discussed in detail here. They may be found by consulting the original papers cited in this article (see also the review by LONGENECKER and DAUBERT (1945) for recently introduced techniques and for leading references in this connection). Attention is, however, directed to advantages which may be gained by using micro and semi-micro procedures for isolation and characterization in the preliminary investigation of lipids of microorganisms. These rapid and economical procedures are of value in making possible surveys of groups of organisms, and in facilitating subsequent large scale preparation of material for biological testing.

A word should be said concerning the reliability of the data in the various tables. The total readily extracted lipids and the phospholipid and acetone-soluble lipid fractions were obtained in each case by the methods described above, or by minor modifications of these methods. A discussion of factors affecting the relative amounts of lipids which may be obtained has been given above; notes to certain of the tables indicate variations in the treatment of the cells prior to extraction. The properties listed for the acetone-soluble lipid and phospholipid fractions have been determined by conventional methods and should, therefore, be directly comparable. The cleavage products from hydrolysis or degradation of the lipid fractions were isolated and characterized by procedures which satisfactorily establish their identity. For details, the original papers should be consulted. The data on yields of these products, in general, represent those amounts of the substances actually isolated, and may be considered to be minimum values.

**Readily Extracted Lipids:**—The lipids extracted from fresh, living cells by means of neutral organic solvents have been designated *readily extracted lipids* to distinguish them from *bound lipids* and *total lipids.* The readily extracted lipids doubtless comprise that fraction of the total lipid material which is held loosely in the cell wall or in parts of the cell structure to which organic solvents can readily penetrate. This fraction may be considered to include lipids which have been synthesized by the cells but which have not become bound by chemical linkage to protein or carbohydrate elements of the cell skeleton together with intermediate substances such as free fatty acids, sterols or other alcohols, and autolytic or hydrolytic products of this type involved in metabolic processes of the cells. The chemically bound lipids, on the other hand, may be considered to represent an integral part of the cell structure, possibly representing a relatively constant fraction of the cells. The limited data available do not suffice to establish this point at present. A precise measure of the total lipids is very difficult to achieve experimentally, since complete liberation of the total amount of lipid material requires procedures which involve extensive lipid degradation. The readily extracted lipids of the fungi so far studied appear to consist of glycerides, phospholipids, free fatty acids, and sterols and are present in amounts dependent in part on the medium and culture conditions employed. The phospholipids are usually separated from the other components of the readily extracted lipids on the basis of their acetone-insolubility. Table 1 gives the amounts of the various lipid fractions isolated from two typical yeast-like, pathogenic fungi and from several non-pathogenic fungi. Comparable data on the readily extracted lipids of certain pathogenic and non-pathogenic bacteria are shown in Table 2.

TABLE 1.—*Amounts of lipid fractions isolated from pathogenic and non-pathogenic fungi:*—

| | *Readily extracted lipids* | | | | |
|---|---|---|---|---|---|
| ORGANISM | *Phospholipids* (%) | *Acetone-soluble lipids* (%) | *Bound lipids* (%) | *Total lipids extracted* (%) | *References*[b] |
| *Candida albicans* ......... | 0.2 | 5.1 | 8.6 | 13.9 | (39) |
| *Blastomyces dermatitidis* ... | 2.3 | 7.2 | 5.7 | 15.2 | (38) |
| *Saccharomyces cerevisiae* .. | 1.8 | 1.3 | not isol. | 3.1 | (33) |
| *Geotrichoides sp.* .......... | 1.9 | 3.2 | 10.4 | 15.5 | (49) |
| *Penicillium*[a] *aurantio-brunneum* .................. | 0.2 | 11.4 | not isol. | 11.6 | (29) |
| *Aspergillus sydowi* ....... | 0.7 | 7.9 | not isol. | 8.6 | (45, 52) |
| *Rhizopus*[a] *japonicus* | 1.2 | 8.5 | not isol. | 9.7 | (30) |

[a] Cells killed before extraction of lipids; extensive hydrolysis may have occurred. All other cells placed in extraction solvents without killing.

[b] Citations at end of article are numbered serially.

The changes in relative amounts of readily extracted lipids of *Blastomyces dermatitidis* brought about by pre-treatment of cells before extraction is clearly shown in Table 3. The total amounts of lipids isolated from the four batches are not significantly different; but the amount of phospholipid fraction in Batch II is very low, evidently due to hydrolytic degradation (possibly enzymatic) to acetone-soluble products. Variation in yields of lipids from several batches of *Aspergillus sydowi* treated in different ways have been reported by STRONG and PETERSON (1934), and by WOOLLEY, STRONG, PETERSON and PRILL (1935).

TABLE 2.—*Amounts of lipid fractions isolated from pathogenic and non-pathogenic bacteria:**—

| | *Readily extracted lipids* | | | | | |
|---|---|---|---|---|---|---|
| ORGANISM | *Acetone-soluble lipids* (%) | *Phospho-lipids* (%) | *Wax* (%) | *Total readily extracted lipids* (%) | *Bound lipids* (%) | *References* |
| *C. diphtheriae*[a] ......... | ca 4.0 | ca 0.5 | Small Amount | ca 5.0 | ... | (13) |
| *Lactobacillus acidophilus* .. | 4.8[b] | 2.2 | ... | 7.0 | ... | (9, 10, 11) |
| *Phytomonas tumefaciens*[c] | | | | | | |
| (*a*) *glycerol medium* ... | 1.1 | 0.9 | ... | 2.0 | ... | (2) |
| (*b*) *sucrose-glutamic acid medium* ........... | 2.2 | 3.8 | ... | 6.0 | ... | (2) |
| *Human Tubercle Bacillus*[c] (*H-37*) ............... | 6.2 | 6.5 | 11.0 | 23.7 | 12.2[d] | (2, 3) |
| *Avian Tubercle Bacilli*[c] (*No. 531*) ........... | 2.2 | 2.3 | 10.8 | 15.3 | 10.8 | (2, 3) |
| *Bacillus leprae*[c] (*No. 370*) | 6.5 | 2.2 | 10.0 | 18.7 | 19.2 | (2, 3) |

* Living cells extracted directly with organic solvents.

[a] Contains unsaturated, branched-chain fatty acids of high molecular weight.

[b] Composed of about 44% free fatty acids, mainly *dihydroxystearic acid,* and about 56% neutral fat.

[c] The lipids contain *liquid saturated fatty acids.*

[d] Bound lipids isolated from a batch of Strain A-10 human tubercle bacilli.

TABLE 3.—*Variation in amounts of readily extracted lipids in Blastomyces dermatitidis* (38):—

| | *Acetone-soluble lipids* | | *Phospholipids* | | *Total extracted lipids* |
|---|---|---|---|---|---|
| BATCH | *% of cells* | *% of lipids* | *% of cells* | *% of lipids* | *% of cells* |
| I* ............ | 7.2 | 75.7 | 2.3 | 24.3 | 9.5 |
| II ............. | 8.2 | 91.8 | 0.7 | 8.2 | 8.9 |
| III ............ | 5.9 | 65.9 | 3.0 | 34.1 | 8.9 |
| IV ............ | 5.6 | 65.7 | 2.9 | 34.3 | 8.5 |

* Batch I was grown on a medium containing blood (10 g. dextrose, 10 g. peptone, 15 g. agar-agar, 3 g. beef extract, 0.5 g. sodium chloride, and 1000 cc. distilled water; to this was added 20 cc. of laked blood--one part blood to three parts distilled water). Batches II, III and IV were grown on the same medium without added blood. Batch II was left for two weeks in a killing solution of 1% aqueous phenol before extraction of lipids, whereas the other batches were placed in alcohol-ether (1:1) immediately after removal of the living cells from the culture medium. The extraction and working up were the same for all four cases.

TABLE 4.—*Constants of the acetone-soluble lipids of pathogenic and non-pathogenic fungi:*—

| ORGANISM | *Iodine number* | *Acid number* | *Saponification number* | *Ester number* | *Reichert-Meissel number* | *Unsaponifiable matter (%)* |
|---|---|---|---|---|---|---|
| *Candida albicans* (39) .......... | 81.0 | 96.3 | 183.9 | 87.6 | .. | 13.6 |
| *Blastomyces dermatitidis* (38) .. | 106.6 | 45.3 | 191.5 | 146.2 | 2.7 | 8.0 |
| *Geotrichoides sp.* (49) .......... | 128.0 | 105.0 | 170.0 | 73.0 | 0.8 | 9.4 |
| *Saccharomyces cerevisiae* (33) ... | 61.3 | 28.6 | 109.6 | 81.0 | 2.3 | 45.6* |
| *Penicillium javanicum* (51) ..... | 84.0 | 10.8 | 191.0 | 180.4 | 0.3 | 2.0 |
| *Aspergillus sydowi* (45) ..... ... | 114.4 | 43.4 | 169.5 | 126.1 | 0.5 | 8.2 |
| *Aspergillus niger* (6) ...... ... | 95.1 | 71.2 | 169.0 | 97.8 | 1.0 | 12.0 |

* Due to a hydrocarbon accidentally introduced during cultivation of the yeast.

*Properties and composition of the acetone-soluble lipids.*—The acetone-soluble lipids constitute the major part of the lipids extracted by means of alcohol and ether from most of the fungi studied up to the present time (*see* Table 1). This lipid fraction is usually yellow-brown to red in color, possessing an odor somewhat characteristic of cultures of the corresponding organism. The constants of the acetone-soluble lipids of *Candida albicans* and *Blastomyces dermatitidis* are given in Table 4 together with the constants of the acetone-soluble lipids of several non-pathogenic fungi. Table 5 gives comparable data on acetone-soluble lipids of a number of bacteria.

TABLE 5.—*Constants of the acetone-soluble lipids of several bacteria:*—

| ORGANISM | *Iodine number* | *Saponification number* | *Acid number* | *Ester number* | *Reichert-Meissel number* | *Unsaponifiable matter (%)* | *References* |
|---|---|---|---|---|---|---|---|
| *Lactobacillus acidophilus (neutral fat)* ....... | 35.9 | 209 4 | .... | .... | 25.2 | 6.7 | (9, 10) |
| *Phytomonas tumefaciens* | 108.5 | 201.2 | 53.2 | 148.0 | .... | 7.1 | (14, 15) |
| *C. diphtheriae* ........ | .... | 176 | 126 | 50 | .... | .... | (13) |
| *Human tubercle bacillus (H-37)* ......... | 52.6 | 203.6 | 60.3 | 143.3 | 3.9 | 10.3 | (2, 3) |
| *Avian tubercle bacillus (No. 531)* .......... | 63.7 | 180.1 | 44.5 | 135.6 | 10.9 | 20.1 | (2, 3) |
| *Bacillus leprae (No. 370) (neutral fat)* ....... | 47.9 | .... | .... | .... | 5 2 | 22.1 | (2, 3) |

All of the acetone-soluble lipid fractions appear to contain small amounts of volatile acids. The iodine numbers show considerable variation, which corresponds roughly to the relative amounts of oleic and linoleic acids present. The variations in the relative amounts of free fatty acids and glycerides indicated by the acid and ester numbers are probably dependent in part on differences in growth conditions.

The chemical composition of the acetone-soluble lipids has usually been determined after saponification of the material with hot alcoholic potassium hydroxide. Table 6 gives the percentage composition of the acetone-soluble lipids of the two pathogenic fungi and of a number of typical non-pathogenic fungi.

TABLE 6.—*Percentage composition of the acetone-soluble lipids of several fungi:—*

| FRACTION | *Blastomyces dermatitidis* (38) | *Candida albicans* (39) | *Geotrichoides sp.* (49) | *Saccharomyces cerevisiae* (33) | *Penicillium aurantiobrunneum* (29) | *Penicillium javanicum* (51) | *Aspergillus sydowi* (45) | *Aspergillus niger* (6) |
|---|---|---|---|---|---|---|---|---|
| Water soluble ........ | 4 | 6.9 | 4.4 | 7 | 10.1 | 6.4 | 11.0 | 20.5 |
| Unsaponifiable ....... | 8 | 13.6 | 9.4 | 45.6* | 4.5 | 2.0 | 8.2 | 12 |
| Fatty acids .......... | 88 | 79.5 | 86.2 | 47.4 | 85.4 | 91.6 | 81.0 | 67.5 |
| Saturated acids ..... | 14.5 | 20.6 | 7.8 | 9.5 | 14.0 | 30.8 | 24.9 | 15.0 |
| Unsaturated acids .... | 73.5 | 58.9 | 78.4 | 37.9 | 71 4 | 60.8 | 56.1 | 52.5 |
| Palmitic acid ......... | 9.7 | 15.5 | 5 0 | 13.5 | 8.6 | 21.4 | 8.8 | 7.1 |
| Stearic acid .......... | 4.8 | 5.1 | 2.8 | 4.5 | 5.3 | 8.6 | 11.0 | 0.9 |
| Tetracosanoic acid ... | Present | ... | ... | ... | Traces | 0.8 | 0.9 | 1.8 |
| Oleic acid ........... | 55.0 | 48.6 | 36.1 | 25.2 | 40.2 | 31.7 | 29.6 | 21.5 |
| Linoleic acid ......... | 18.5 | 10.3 | 42.3 | 10.7 | 31 2 | 29.1 | 16.3 | 23.9 |
| Glycerol ............ | Present | Present | Present | 5.9 | Present | Present | Present | 6.2 |
| Sterols ...... ...... | 4 | 7 | Present | Present | 1.9 | Present | 5.4 | 1.4 |

* In part due to hydrocarbon contaminant.

The saturated fatty acid fractions from the acetone-soluble lipids of all of the fungi examined contained palmitic and stearic acids; in several, tetracosanoic acid was also present. The unsaturated fatty acid fractions from all of these organisms consisted essentially of oleic acid and linoleic acid in varying proportions. The ratio of oleic acid to linoleic acid was higher in the pathogenic species. Only traces of more highly unsaturated acids were observed. Sterols were present in all of the unsaponifiable fractions and comprised roughly half of the unsaponifiable material in the case of the two pathogenic fungi. Ergosterol was identified in the case of both pathogenic and non-pathogenic organisms. The water-soluble fraction in every case contained glycerol.

It is evident that the acetone-soluble lipids of both the pathogenic and non-pathogenic fungi contain neutral glycerides, free fatty acids, and sterols. The sterols probably occur both free and as fatty acid esters. A considerable part of the unsaponifiable fraction in the case of every organism has not been characterized. The differences observed between the acetone-soluble lipids of the pathogenic fungi and those of the non-pathogenic organisms appear to be quantitative rather than qualitative.

*Properties and composition of the phospholipids.*—The phospholipids of *Blastomyces dermatitidis* and *Candida albicans* have been investigated in some detail. These preparations were obtained from alcohol-ether extracts of living cells, by methods described in the previous section. The phospholipids are readily soluble in ether, chloroform, and benzene and are relatively insoluble in acetone and alcohol.

They are easily dispersed in water, forming slightly turbid colloidal solutions. Addition of acids or salts to the concentrated aqueous solutions causes partial coagulation. Table 7 summarizes certain properties of the two preparations. That the physical properties of these phospholipids differ somewhat from those of several non-pathogenic fungi is illustrated by comparable data included in the table.

The chemical composition of the phospholipid fraction from *Blastomyces dermatitidis* was determined after hydrolysis with hot 5% aqueous sulfuric acid. Table 8 summarizes the data obtained. Some data on the phospholipid from *Candida albicans* are also included in this table, together with comparable data on the phospholipids of certain other typical non-pathogenic fungi.

The data in Table 8 show a great similarity between the phospholipid fractions from *Blastomyces dermatitidis* and *Saccharomyces cerevisiae*. The presence of choline, ethanolamine, and glycero-phosphoric acid in the hydrolysates of both phospholipid preparations indicates that both lecithin and cephalin are present in each case. One qualitative difference is noted: the presence of small amounts of a carbohydrate in the water-soluble fractions from the phospholipid of *Blastomyces dermatitidis*. This would indicate the presence of a carbohydrate-containing phospholipid in addition to lecithin and cephalin. No claim for homogeneity in the chemical sense can be made for the phospholipid fractions, since these fractions are amorphous, and since they are purified simply by reprecipitation from ether by means of acetone. It is therefore only to be expected that the phospholipid fractions represent mixtures of two or more individual phospholipids, the solubility properties of which are fairly similar. The ratio of the fatty acids to one another also indicates the presence of several phospholipids differing only in the fatty acid residues present in the molecule.

It is evident from the foregoing discussion that the phospholipid from *Blastomyces dermatitidis* is a mixture composed of lecithin, cephalin, and carbohydrate-containing types of phospholipids. Tables 7 and 8 include data on three phospholipid preparations obtained from a non-pathogenic fungus, *Geotrichoides sp.*, which show that this organism also produces carbohydrate-containing phospholipids. The phospholipids from acid-fast bacteria are predominantly of the latter type, no cephalin or lecithin having been conclusively demonstrated as components. Like the phospholipids from the human tubercle bacillus, which produce extensive tissue reactions characteristic of tuberculosis when injected into animals (SABIN, 1932), the phospholipids of *Blastomyces dermatitidis* give rise to tissue reactions simulating those encountered in human blastomycosis (BAKER, 1942).

Two isomeric structures may be written for lecithin, as shown in Figure 1. The isomerism depends upon the position of the linkage between the glycerol and phosphoric acid residues. Since the β-lecithin

Table 7.—*Properties of the phospholipids:*—

| | *Phospholipid from* | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Geotrichoides sp.* (49) | | | | |
| Properties | *Blastomyces dermatitidis* (38) | *Candida albicans* (39) | Fraction A | Fraction B | Fraction C | *Aspergillus sydowi* (52) | *Saccharomyces cerevisiae* (34, 44) |
| Color | Buff | Cream | Yellowish | Yellowish | Yellow-brown | Light brown | Brownish |
| Consistency | Powder | Powder | Solid | Solid | Sticky | Powder | Brittle solid |
| Hygroscopicity | Medium | Fairly high | Low | Medium | Medium | Low | High |
| Melting point | 100°-125° C. | 105°-125° C. | 187°-189° C. | 161°-165° C. | 160°-163° C. | Not given | Not given |
| Phosphorus | 3 89% | 3.97% | 3.04% | 3.41% | 3.39% | 3.78% | 4.22% |
| Nitrogen (total) | 1.78% | 2.18% | 1.41% | 1.37% | 1.42% | 1.68% | 1.78% |
| Nitrogen (amino) | Not det. | Not det. | 0.515% | 0.822% | 0.788% | ........ | Approx. 0.53% |
| P.N ratio | 1.1.01 | 1:1.21 | 1:1.03 | 1:0.89 | 1:0.93 | 1:0.98 | 1:0.98 |
| $NH_2$-N: Total N ratio | Not det. | Not det. | 1:3.7 | 1: 6.0 | 1:5.6 | Approx. 1:1 | Approx. 1:3.4 |
| Iodine number | 64 | Not det. | 27.8 | 55.2 | 69.5 | Not reported | Not reported |
| Molisch test (aq soln.) | Slight positive | Slight positive | Positive | Slight positive | Negative | Not reported | Negative |

contains an asymmetric carbon atom, the isolation of an optically active glycerophosphoric acid fraction on hydrolysis of the lecithin will establish the presence of a β-lecithin. In the case of carbohydrate-containing phospholipids or other phospholipids derived from α- and β-glycerophosphoric acid, the biological properties may depend to a considerable degree upon the isomer studied. That the same situation obtains with cephalin is illustrated in Figure 2. No data are available on the lecithin and cephalin fractions from *Blastomyces dermatitidis* or *Candida albicans* which afford information on the isomers present. The lecithin fraction from *Saccharomyces cerevisiae,* however, does appear (SALISBURY and ANDERSON, 1936) to be composed of a mixture of α- and β-lecithins. The evidence indicates that the α-form predominates in about a 4:1 ratio. The cephalin fraction from the latter organism yielded only optically inactive glycerophosphoric acid on hydrolysis; it would thus appear to contain only α-cephalin.

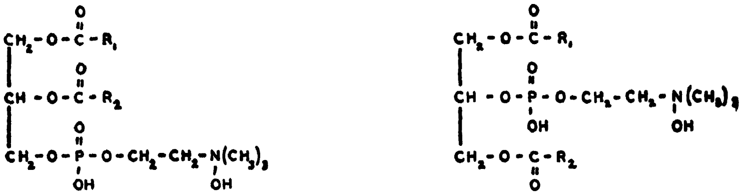
TABLE 8.—*Percentage composition of the phospholipids:*—

| FRACTION | *Blastomyces dermatitidis* (38) | *Candida albicans* (39) | *Geotrichoides sp.* (49) | *Saccharomyces cerevisiae* (34, 44)[b] | *Aspergillus sydowi* (52)[c] |
|---|---|---|---|---|---|
| Water-soluble | 32 | not det. | 37 | 31 | 38 |
| Unsaponifiable | 3 | not det. | 25[a] | 3 | 0.4 |
| Fatty acids | 65 | not det. | 38 | 66 | 62 |
| Saturated acids | 12 | not det. | similar to those of acetone-soluble lipids | 10 | 9 |
| Palmitic acid | 8 | not det. | | 5 | 4.5 |
| Stearic acid | 4 | not det. | | 5 | 4.5 |
| Unsaturated acids | 53 | not det. | | 56 | 53 |
| Oleic acid | 49 | not det. | | 51 | 51 |
| Linoleic acid | 4 | not det. | | traces | 2 |
| Glycerophosphoric acid | 10 | present | not det. | present | present |
| Choline | present | not det. | not det. | present | present |
| Ethanolamine | present | not det. | not det. | present | present |
| Carbohydrates | small amount | small amount | 25 | absent | (absent ?) |

[a] Insoluble in water, ether, and cold alcohol; soluble in hot alcohol. Fraction A used in hydrolysis.
[b] Composed of lecithin:cephalin in the approximate ratio 4:1.
[c] Lecithin:cephalin appeared to be roughly 1:1.

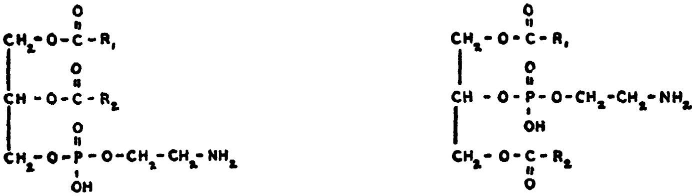
Brief mention is made of acetal phospholipids (LONGENECKER and DAUBERT, 1945), Figure 3-A, in which only one isomeric form of glycerophosphoric acid is possible. Acetal phospholipids have not as yet been found in lipids of fungi, but represent a possible type of component. Carbohydrate-containing phospholipids (Figure 3-B) in which two isomers due to the glycerophosphoric acid moiety are possible, may also have isomeric forms as a result of the asymmetry of the carbohydrate portion. A more complete investigation of the phospholipids of various pathogenic and non-pathogenic microorganisms would appear to be profitable.

*Wax-like fractions.*—A well-characterized wax, ergosteryl palmitate, m.p. 107°-108° has been isolated from a number of organisms of the *Penicillium* group (OXFORD and RAISTRICK, 1933).



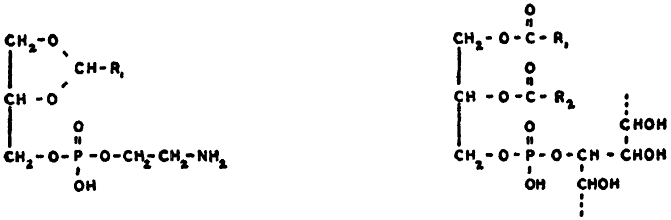
α-LECITHIN β-LECITHIN

FIGURE 1



α-CEPHALIN β-CEPHALIN

FIGURE 2



A B

FIGURE 3

From the acetone-soluble liquids of the yeast-like pathogenic fungus, *Blastomyces dermatitidis,* there has been isolated* a fraction resembling a soft wax. When 65.5 g. of crude acetone-soluble lipids of *Blastomyces dermatitidis* in 175 cc. of acetone was mixed with 75 cc. of methanol, an oily layer separated. On cooling overnight in a refrigerator, lumps of yellow wax-like solid separated from the oily insoluble layer. The yellow substance (2.79 g. recovered) was isolated by centrifuging the cold solution, decanting the two supernatant layers, and washing the residual wax-like material with cold methanol. The residue was dissolved in 50 cc. of cold acetone and reprecipitated with 50 cc. of cold methanol several times. The substance thus obtained was a solid at 10° C. and a very thick, pale yellow oil at room temperature; it contained no nitrogen, phosphorus, or ash. It had an iodine number of 97.

For determination of the chemical composition, a sample was saponified by refluxing with 4% alcoholic potassium hydroxide for twelve hours under nitrogen. Unsaponifiable material, fatty acids, and water soluble material were isolated by conventional methods. The water-soluble fraction, 2.8 per cent of the wax, was characterized as nearly pure glycerol by its conversion to glycerol tribenzoate, m.p. 75°-76° C., in good yield (EINHORN and HOLLANDT, 1898).

The fatty acids, 89.8 per cent of the wax, were separated by the TWITCHELL (1921) method into solid and liquid fatty acids. The solid fatty acid fraction was a white crystalline solid representing 21.4 per cent of the total fatty acids. It was completely saturated. Recrystallized twice from acetone, it was obtained in the form of shining plates which melted at 80°-82° C., and had a neutral equivalent of 370.2. The melting point and neutral equivalent are close to the values for n-tetracosanoic acid (m.p. 83°-84° C., N. Eq., 368); thus it appears probable that the saturated acids of the wax-like fraction of *Blastomyces dermatitidis* are composed mainly of n-tetracosanoic acid.

The liquid acids represented 68.4 per cent of the wax. This fraction had an iodine number of 135. Bromination of a sample weighing 1.540 g. gave 0.7830 g. of tetrabromostearic acid. The latter was characterized by its melting point, 113°-114° C. (which was not depressed by admixture with authentic tetrabromostearic acid), and by bromine analysis (calculated: Br, 53.29, found: Br, 53.01). The remainder of the bromination product appeared to be dibromostearic acid. No evidence was obtained for the presence of fatty acids more highly unsaturated than linoleic. The unsaturated fatty acids therefore consist only of oleic and linoleic acids. Oleic acid forms 30 per cent and linoleic acid 38 per cent of the wax.

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* Previously unpublished results.

TABLE 9.—*Lipids extracted from 36.32 g. dry weight of cells of Blastomyces dermatitidis previously extracted with alcohol-ether and with chloroform:*—

| | *Fraction* | | | *Sub-fractions* | | *Per cent of dry wt. of cells* | |
|---|---|---|---|---|---|---|---|
| SOLVENT | *No.* | *Wt. g.* | *No.* | *properties* | *Wt. g.* | | |
| Boiling ethyl alcohol | I | 1.016 | I-a | Insoluble in cold alcohol | 0.106 | 0.29 | 2.72 |
| | | | I-b | Soluble in cold alcohol. Insoluble in ether | 0.035 | 0.10 | |
| | | | I-c | Ether-soluble | 0.8450 | 2.33 | |
| Boiling ether | II | 0.030 | | | | | 0.08 |
| Boiling ethyl alcohol containing 1% HCl | III | 0.2130 | | (Ether-soluble fraction after evaporation to dryness *in vacuo* and extraction with ether.) | | | 0.59 |
| Boiling alcohol-ether (1:1) containing 1% HCl | IV | 0.0768 | | (Ether-soluble; treated similarly to Fraction III.) | | | 0.21 |
| Boiling ethyl alcohol containing 2% KOH | V<br>(Ether-soluble) | 0.0344 | V-a | Unsaponifiable | 0.0241 | | 0.09 |
| | | | V-b | Fatty acids | 0.0103 | | |
| Total extracted lipids | | | | | | | 3.69 |

The unsaponifiable fraction was shown to contain about 45 per cent of sterols by colorimetric determination using the BLOOR (1916) method with ergosterol as standard. Some other higher alcohols were present, but these have not been characterized.

**Bound Lipids**:—The bound lipids of *Candida albicans* and *Blastomyces dermatitidis* have been investigated in some detail (PECK and HAUSER, 1940). Tables 9 and 10 summarize the amounts of bound lipids isolated from these organisms.

After extraction of the lipid fractions shown in Tables 9 and 10, the cells of *Blastomyces dermatitidis* still contained 2.0 per cent of

TABLE 10.—*Lipids extracted from 29.19 g. dry weight of cells of Candida albicans previously extracted with alcohol-ether and with chloroform:*—

| | *Fraction* | | | *Sub-fractions* | | *Per cent of dry wt. of cells* | |
|---|---|---|---|---|---|---|---|
| SOLVENT | *No.* | *Wt. g.* | *No.* | *properties* | *Wt. g.* | | |
| Boiling ethyl alcohol | I | 0.9983 | I-a | Insoluble in cold alcohol | 0.277 | 0.95 | 3.42 |
| | | | I-b | Soluble in cold alcohol. Insoluble in ether | 0.057 | 0.20 | |
| | | | I-c | Ether-soluble | 0.6643 | 2.28 | |
| Boiling ether | II | Traces only | | | | | |
| Boiling ethyl alcohol containing 1% HCl | III | 0.2230 | | (Ether-soluble fraction after evaporation to dryness *in vacuo* and extraction with ether.) | | | 0.76 |
| Boiling alcohol-ether containing 1% HCl | IV | .0954 | | (Ether-soluble; treated similarly to Fraction III.) | | | 0.33 |
| Boiling alcohol containing 2% KOH | V | 0.0378 | V-a | Unsaponifiable | 0.0296 | | 0.13 |
| | | | V-b | Fatty acids | 0.0082 | | |
| Total extracted lipids | | | | | | | 4.64 |

lipids and the cells of *Candida albicans* 4.0 per cent, as determined by solution in 6 *N* hydrochloric acid and extraction with ether (TURPEINEN, 1935, 1936).

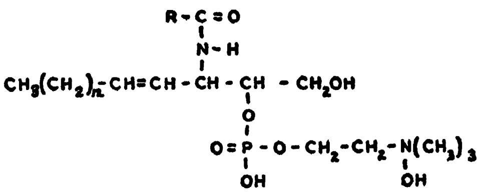
Fraction I represents material extracted from the cells with boiling alcohol. This is composed partly of bound lipids, partly of certain lipids soluble only in hot alcohol, and a small amount of mechanically bound lipids of the type extracted by alcohol-ether mixture. From the hot alcohol extracts, on cooling to room temperature, a white powder separated. In Table 11 are given certain analytical data obtained on purified samples of this fraction from the two organisms. This material was insoluble in ether, cold alcohol, water, and cold acid or alkaline aqueous solutions. It was soluble in hot alcohol and in pyridine. From the latter solvent, it could be reprecipitated by the addition of one volume of acetone. These substances were unsaturated, since they absorbed bromine.

TABLE 11. — *Data on lipids soluble in hot alcohol and insoluble in cold alcohol:* —

| ORGANISM | *Per cent of nitrogen* | *Per cent of phosphorus* | *N:P* | *Molisch test* |
|---|---|---|---|---|
| *Blastomyces dermatitidis* .... | 3.00 | 2.55 | 2.6 | + |
| *Candida albicans* ............ | 1.21 | 1.38 | 1 9 | + |

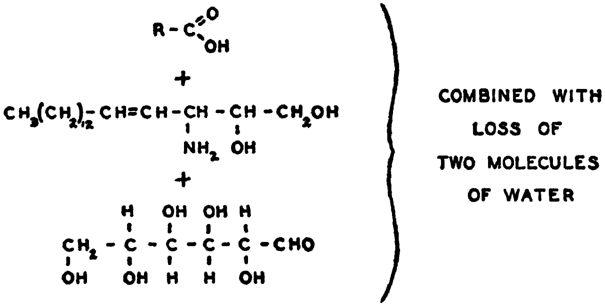
Saponification of the substance insoluble in cold alcohol gave fatty acids and a water-soluble fraction containing carbohydrate, phosphoric acid, and nitrogenous substances. Further characterization of these components has not been carried out, but the data available on this fraction indicate a similarity to sphingomyelin, Figure 4, and the cerebrosides, Figure 5, which often occur together and which are difficult to separate in pure condition. Sphingomyelin types usually contain approximately four per cent each of nitrogen and phosphorus, the N:P ratio being 2:1. Carbohydrates, however, are not present. The cerebrosides contain a carbohydrate moiety and approximately two per cent of nitrogen, but no phosphorus.

A compound, $C_{46}H_{93}NO_5$, designated fungus cerebrin has been isolated from yeast by REINDEL *et al.* (1940), and may be considered to be a bound lipid. The structure shown in Figure 6 was postulated for this substance. More recently, the same type of substance was isolated from *Aspergillus sydowi* by BOHONOS and PETERSON (1943) after autolysis had occurred. Analyses on the latter product and on its tetraacetate yielded the same empirical formula, $C_{46}H_{93}NO_5$, for the free compound. Degradation studies led to the conclusion that this material was identical with the fungus cerebrin previously isolated from yeast. The necessity for autolysis prior to extraction of fungus cerebrin from both organisms suggests that it exists in the cells in a tightly bound condition.



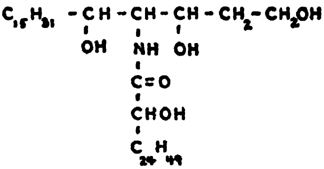
SPHINGOMYELIN

FIGURE 4



CEREBROSIDE

FIGURE 5



FUNGUS CEREBRIN

FIGURE 6

Sub-fraction I-b from both organisms (Tables 9 and 10) was a saturated, buff-colored product containing about 7 per cent of nitrogen and only traces of phosphorus. It was slightly soluble in water. Saponification gave fatty acids, a polysaccharide, and water-soluble nitrogenous compounds. These fractions have not been further characterized.

Sub-fractions I-c and Fractions III and IV from both organisms (Tables 9 and 10) were brown oils similar to the acetone-soluble lipids extracted from the fresh cells with alcohol-ether. The iodine numbers of the fractions ranged between 70 and 90. Only traces of phosphorus and nitrogen were present. Saponification gave fatty acids, glycerol, traces of carbohydrates, and unsaponifiable material containing sterols. The relative amounts of fatty acids and unsaponifiable material obtained on saponification are shown in Table 12.

TABLE 12.—*Percentages of fatty acids and unsaponifiable material in bound lipid fractions:*—

| ORGANISM | *Fraction* | *Fatty acids per cent* | *Unsaponifiable material per cent* |
|---|---|---|---|
| *Blastomyces dermatitidis* .......... | I-c | 36.5 | 58.5 |
| | III | 30.6 | 65.7 |
| | V | 29.9* | 70.1* |
| *Candida albicans* ........ .. ...... | I-c | 60.1 | 23.2 |
| | III | 52.6 | 44.0 |
| | V | 21.7* | 78.3* |

* Calculated on the basis of total ether extract.

There is considerably more unsaponifiable material in most of the bound lipid fractions than in the acetone-soluble fractions of the easily-extracted lipids. The unsaponifiable fractions from the bound lipids were obtained as brown oils containing crystals of sterols. In the mixed unsaponifiable fractions from *Candida albicans,* there was found 20.4 per cent of sterol, whereas in the corresponding fractions from *Blastomyces dermatitidis* there was found 8.4 per cent.

The fatty acids from the bound lipids had iodine numbers ranging between 90 and 100. There was obtained 40 per cent of saturated acids from *Blastomyces dermatitidis* and about 45 per cent from *Candida albicans.* The unsaturated fatty acid fractions were composed essentially of oleic and linoleic acids.

**Miscellaneous Components of the Lipid Fractions:**—Pigments are present in the lipids of all pathogenic and non-pathogenic fungi, but in only a few instances have these substances been isolated or characterized. Strains of the pathogenic yeast-like fungus, *Candida albicans,* and of the relatively non-pathogenic related organism, *Candida stellatoidea,* have been shown by JONES and PECK (1940) to produce a green pigment which is soluble in acetone, ethyl acetate, and acetic acid and practically insoluble in ether and methyl, ethyl, and amyl alcohols. This pigment exhibits an absorption band at about 6550Å in ethyl acetate solution. *Candida stellatoidea* produces about ten times as much of the pigment as *Candida albicans.* The pigment is an indicator, being blue-green in acid solution and yellow in alkaline solution. It is unstable in the presence of oxidizing or reducing reagents.

Of known types of pigments which have been shown to be present in lipid fractions obtained from fungi, carotinoids and quinones may be mentioned. It is also possible that antibiotic substances may be present in the lipid fractions obtained from pathogenic and non-pathogenic fungi. Recent work has demonstrated the wide-spread production of antibiotic substances by fungi. The solubility properties of a number of these substances are such that they could be carried along with the lipid fraction, and indeed might be present in the cells in close association with lipids. The classic example in the case of bacteria is pyocyanase, the crude lipid of *Pseudomonas aeruginosa,* which contains much antibiotic material.

**Discussion:**— In this survey, attention has been focused mainly on the lipids of two pathogenic, yeast-like fungi, *Blastomyces dermatitidis* and *Candida albicans,* which appear to be representative of a fairly considerable group of pathogenic microorganisms. The lipids of these two fungi have been shown to be essentially the same as far as qualitative composition is concerned. Neutral glycerides, sterol esters, free fatty acids and sterols, lecithin and cephalin types of phospholipids, and small amounts of carbohydrate-containing phospholipids represent the major constituents of the readily extracted lipids. The bound lipids, on the other hand, appear to contain considerable amounts of more complex substances, including fractions resembling the sphingomyelins and cerebrosides. The differences observed between the lipids of the two organisms are mainly quantitative.

The readily extracted lipids of the non-pathogenic fungi show qualitative similarity to the corresponding lipid fractions from the two pathogenic forms. The presence of carbohydrate-containing phospholipids in the pathogenic group and the absence of this type of phospholipid in all but one of the non-pathogenic fungi constitutes, perhaps, the most significant variation in the readily extracted lipid fractions. The phospholipids of a number of pathogenic bacteria, particularly the acid-fast bacteria, contain polysaccharides (1, 2, 3, 13). These polysaccharides have been shown in many cases to be immunologically specific, and may be intimately concerned with phases of the disease processes.

Insufficient data are available to permit comparisons between bound lipid fractions of pathogenic and non-pathogenic fungi, but it appears reasonable to suppose that considerable similarity exists among these forms. It is believed possible that the bound lipids, also, may be concerned with the disease processes, since these fractions must be liberated in the tissues when lysis of the invading organisms occurs in the animal body. The capacity of host tissue cells for removal of such breakdown products can doubtless be influenced markedly by various properties of the lipid constituents themselves. The enzyme-inhibiting power of certain lipid types has already been mentioned. During lysis of killed pathogenic organisms in host tissues, there may also be liberated enzymes capable of modifying otherwise harmless lipids (as well as other substances). For example, there might thus be set free a lecithinase which would convert lecithin to the very cytolytic lysolecithin and thereby interfere with host cells endeavoring to remove the dead pathogenic organisms.

The lipids of a number of pathogenic bacteria have been shown (1, 2, 3, 13) to contain considerable amounts of a new series of branched-chain fatty acids of fairly high molecular weight. These unusual fatty acids, when injected into normal animals, stimulate tissue reactions (formation of monocytes, epithelioid cells, and giant cells) similar to those produced in the animals by the corresponding pathogenic organisms. Acids of this type have not so far been observed among the cleavage products of the lipids of any of the fungi studied. It is possible, however, that small amounts of these substances may be present. Much work remains to be done on the fractionation and characterization of the cleavage products of lipids of pathogenic fungi, and likewise on the pathology of these substances. It is not impossible that in such fungi there may be present complex lipid fractions (inactive after hydrolysis or other chemical modification) which in the natural state are capable of eliciting pathologic responses by reason of their chemical structure, specific linkages, or particular configuration.

There appear to be significant differences of a qualitative nature between the lipids of bacteria and fungi. The differences in the composition of the lipids of the various fungi studied are of a less striking nature. There is a great need for further data on the qualitative and quantitative nature of the lipids of both pathogenic and non-pathogenic organisms.

*Literature Cited: —*

1. ANDERSON, R. J. Physiol. Rev. *12:* 166, 1932.
2. ANDERSON, R. J. The chemistry of the lipoids of the tubercle bacillus and certain other microorganisms. Fortschritte der Chemie organischer Naturstoffe. L. Zechmeister, Wien, III Band, 145 (1939).
3. ANDERSON, R. J. The Harvey Lectures, Series *35:* 271, 1939-40.
4. ANDERSON, R. J., R. E. REEVES, and F. H. STODOLA. J. Biol. Chem. *121:* 649, 1937.
5. BAKER, R. D. Am. J. Pathol. *18:* 463, 1942.
6. BERNHAUER, K. and G. POSSELT. Biochem. Z. *294:* 215, 1937.
7. BLOOR, W. R. J. Biol. Chem. *24:* 227, 1916.
8. BOHONOS, N. and W. H. PETERSON. J. Biol. Chem. *149:* 295, 1943.

9. Crowder, J. A. and R. J. Anderson. J. Biol. Chem. *97:* 393, 1932.
10. Crowder, J. A. and R. J. Anderson. J. Biol. Chem. *104:* 399, 1934.
11. Crowder, J. A. and R. J. Anderson. J. Biol. Chem. *104:* 487, 1934.
12. Crowder, J. A., F. H. Stodola, M. C. Pangborn, and R. J. Anderson. J. Am. Chem. Soc. *58:* 636, 1936.
13. Chargaff, E. Zeit. f. Physiol. Chem. *218:* 223, 1933.
14. Chargaff, E. and M. Levine. Proc. Soc. Exp. Biol. Med. *34:* 675, 1936.
15. Chargaff, E. and M. Levine. J. Biol. Chem. *124:* 195, 1938.
16. Czapek, F. Biochemie der Pflanzen. Jena, 3rd ed., 1922.
17. Dubos, R. The Bacterial Cell. Harvard U. Press, Cambridge, 1945.
18. Einhorn, A. and F. Hollandt. Ann. Chem. *301:* 101, 1898.
19. Gerstl, B. and R. Tennant. Yale J. Biol. Med. *14:* 29, 1941.
20. Gersti, B. and R. Tennant. Ann. Rev. Tuberc. *46:* 600, 1942.
21. Gerstl, B. and R. Tennant. Yale J. Biol. Med. *16:* 1, 1943.
22. Gerstl, B., R. Tennant, and O. Pelzman. Yale J. Biol. Med. *17:* 455, 1945.
23. Heidelberger, M. Physiol. Rev. *7:* 107, 1927.
24. Heidelberger, M. Chem. Rev. *24:* 323, 1939.
25. Herrell, W. E. Penicillin and other antibiotic agents. W. B. Saunders, Philadelphia, 1945.
26. Höber, R. The physical chemistry of cells and tissues. Blakiston, Philadelphia, 1945.
27. Jobling, J. W. and W. Petersen. J. Exp. Med. *19:* 239, 1914.
28. Jones, C. P. and R. L. Peck. J. Bact. *39:* 605, 1940.
29. Kroeker, E. H., F. M. Strong, and W. H. Peterson. J. Am. Chem. Soc. *57:* 354, 1935.
30. Lim. J. Fac. Agr. Hokkaido Imp. Univ. *37:* 165, 1929.
31. Longenecker, H. E. and B. F. Daubert. Ann. Rev. Biochem. *14:* 113, 1945.
32. Marrack, J. R. The chemistry of antigens and antibodies. H. M. Stationery Office, Medical Res. Council, London, 1938.
33. Newman, M. S. and R. J. Anderson. J. Biol. Chem. *102:* 219, 1933.
34. Newman, M. S. and R. J. Anderson. J. Biol. Chem. *102:* 229, 1933.
35. Oxford, A. E. and H. Raistrick. Biochem. J. *27:* 1176, 1933.
36. Parpart, A. K. and A. J. Dzieman. Cold Spring Harbor Symp. *8:* 17, 1940.
37. Peck, R. L. J. Am. Chem. Soc. *64:* 487, 1942.
38. Peck, R. L. and C. R. Hauser. J. Am. Chem. Soc. *60:* 2599, 1938.
39. Peck, R. L. and C. R. Hauser. J. Am. Chem. Soc. *61:* 289, 1939.
40. Peck, R. L. and C. R. Hauser. J. Biol. Chem. *134:* 403, 1940.
41. Peterson, W. H., L. M. Pruess, H. J. Gorcica, and H. C. Green. Ind. Eng. Chem. *25:* 213, 1933.
42. Reindel, F., A. Weickmann, S. Picard, K. Luber, and P. Turula. Ann. Chem. *544:* 116, 1940.
43. Sabin, F. R. Physiol. Rev. *12:* 141, 1932.
44. Salisbury, L. F. and R. J. Anderson. J. Biol. Chem. *112:* 541, 1936.
45. Strong, F. M. and W. H. Peterson. J. Am. Chem. Soc. *56:* 952, 1934.
46. Thomas, R. M. and F. I. Dessau. Yale J. Biol. Med. *12:* 185, 1939-40.
47. Twitchell, E. Ind. Eng. Chem. *13:* 806, 1921.
48. Turpeinen, O. Suomen Kemistilehti *8B:* 17, 1935.
49. Turpeinen, O. Ann. Acad. Sci. Fennicae *A46:* 110 pp., 1936.
50. Ueyi, N. and R. J. Anderson. J. Biol. Chem. *94:* 653, 1932.
51. Ward, E. W. and G. S. Jamieson. J. Am. Chem. Soc. *56:* 973, 1934.
52. Woolley, D. W., F. M. Strong, W. H. Peterson, and E. A. Prill. J. Am. Chem. Soc. *57:* 2589, 1935.
53. Zellner, J. Chemie der Höheren Pilze. Wilhelm Engelmann, Leipzig, 1907.
54. Zinsser, H. and S. Bayne-Jones. Textbook of Bacteriology. D. Appleton-Century, New York, 8th ed., 1939.

## *Chapter 12*

# RESPIRATION AND FERMENTATION OF PATHOGENIC FUNGI

*by*

WALTER J. NICKERSON

**Introduction:**— The number of microorganisms on which studies of oxidative activities has been made increases each year, but of the fairly large number of fungi pathogenic for man only a few have been investigated. Our knowledge of cellular oxidation processes has, in large part, been developed through intensive work on a few mammalian tissues, a few bacteria, and a few yeasts. Extension of findings made with these well-studied forms to other organisms has been possible in many instances, but the complexities of mechanisms of cellular oxidation permit possible variations in pathways leading from substrate to final products. For most of the pathogenic fungi we are ignorant of their nutrition, let alone the catabolic paths of oxidation of those substrates. Investigations of the metabolism of fungi causing deep mycoses are few and particularly needed in view of the high mortality occasioned by many organisms in this group.

In the search for more active chemotherapeutic agents for the control of these organisms, work on inhibition of their respiration may prove of value. Substances presently employed in the therapy of fungus infections have seldom been subjected to an analysis of their mode of action; some of these substances may act as respiratory inhibitors. With pathogenic organisms, other than fungi, investigations on the mechanism of therapeutic action of active substances have frequently brought to light more favorable related agents than the substance prompting the analysis. WELCH (1945) in a stimulating review on the relation between chemical structure and biological action of chemotherapeutic substances, points out that the purposeful design of improved active compounds can be undertaken.

Ideally, substances employed in therapy will attack the parasite through some weak link leaving the cells of the host relatively unaffected. Frequently the weak links in the parasite occur in some metabolic process not prominently displayed by cells of the host. Fundamental investigations of all phases of the physiology of the organism are necessary for a foundation to any thorough-going program aimed at the prevention, prophylaxis, and treatment of fungus diseases.

It is to be understood that the respiratory enzyme systems of the pathogens are but one site of attack in the chemotherapeutic approach. And it should be realized that inhibition of oxygen consumption may be an insignificant effect on cells of an organism that can employ hydrogen acceptors other than oxygen for the main part of its metabolism. SEVAG, RICHARDSON and HENRY (1945) have emphasized this contingency as a result of their work on the mode of action of sulfonamides on the respiration of bacteria.

This discussion will be limited to two groups of pathogenic organisms for which information is available. A comparative treatment will be attempted for the pathogenic forms and related non-pathogenic organisms. Yeast-like forms, such as *Candida,* appear to have much in common in their metabolic aspects with non-pathogenic yeasts, while the dermatophytes may be compared with other filamentous fungi.

It is not believed necessary to enter into any review of the mechanisms of cellular respiration that have been uncovered in recent years. For such, reference may be made to the recent reviews by LIPMANN (1943), GREEN and STUMPF (1944), LARDY and ELVEHJEM (1945), to the work of GREEN (1940), the Symposium on Respiratory Enzymes (1942) and to GODDARD (1946).

## YEAST-LIKE FUNGI

### *I. Candida*

The study of gaseous exchange in *Candida* has been employed by nearly all students of the group as a taxonomic tool in the form of comparative qualitative observations on the anaerobic decomposition of carbohydrates by the various species. MARTIN, JONES, YAO and LEE (1937) and MARTIN and JONES (1940) presented a résumé of the utility of such studies, of the difficulties involved in securing reproducible results, and of the procedures yielding reproducible results in their hands. A glance at these may be instructive.

The procedures used by these workers at the Duke University Hospital have yielded uniform results, with hundreds of different isolates of *Candida,* that conform precisely with data from pathogenicity tests and morphological examination. Especially emphasized in their technique are: (*1*) the inoculum must be from a second or third subculture on a glucose-free medium, (*2*) the fermentation broth tubes must be sealed with vaseline, since isolates of *Candida parakrusei* regularly produce acid from sucrose and galactose (under the more aerobic conditions; a possible explanation is given later) if vaseline seals are omitted, but form no acid from either carbohydrate anaerobically, (*3*) the beef-extract fermentation broth must be accurately titrated to *p*H 7.2—an increase of 0.2 *p*H will inhibit the production of acid by *C. albicans* from sucrose and galactose and prevent the formation of both acid and gas from glucose by *C. parakrusei,* (*4*) results are reported after 10 days' incubation at 37° C. The findings for *C. albicans* were based on almost one hundred strains identified from a total of over five hundred isolates of *Candida* and, with the exception of one dissociated strain, all isolates of *C. albicans* were pathogenic for rabbits in 2 to 7 days.

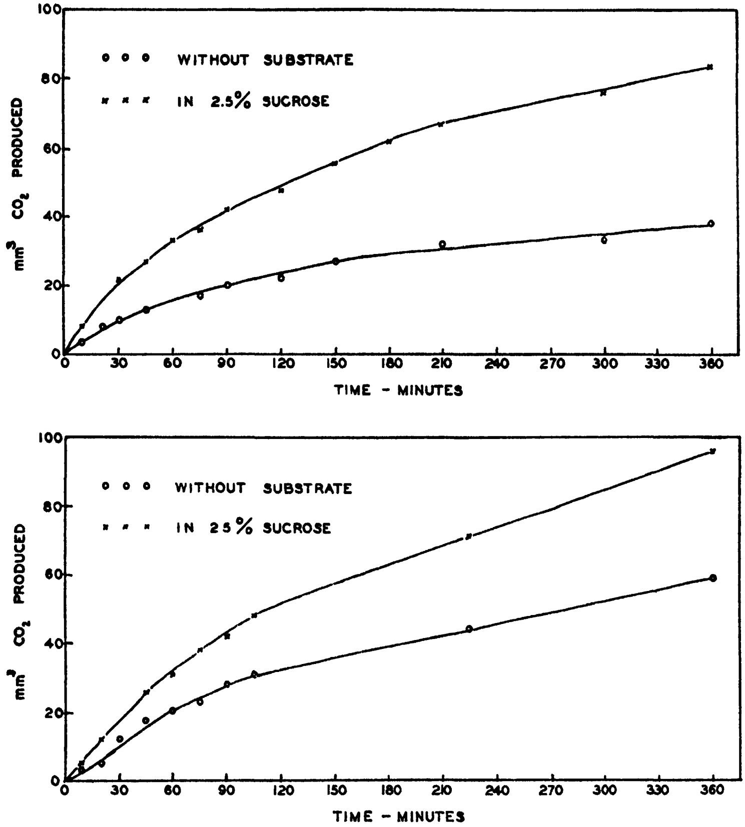


Figure 1. — Anaerobic carbon dioxide production from sucrose and without substrate ("autofermentation") by *Candida albicans* (upper graph) and *Candida parakrusei*. A weak, but positive, fermentation of sucrose is demonstrated for each species. Note the pronounced, steady evolution of gas by each species in the absence of added substrate. (Kluyver and Custers, 1940.)

The results obtained for the decomposition of a given carbohydrate in such large-scale, carefully performed tests possess a high degree of validity, especially when the correlations with pathogenicity and morphology are included. Of course, the observations are qualitative and less sensitive for the detection of gas production than quantitative studies. Quantitative investigations of the fermentation by *C. albicans* are desirable, but due regard must be had for the origin and culture history of the strain used, *p*H at which conducted, temperature and duration of the investigations, and realization of the fact that rapid growth takes place in the procedures of MARTIN *et al.* since a favorable nitrogen and bios nutrition is provided.

Some data from quantitative studies by KLUYVER and CUSTERS (1940) are available for two species of *Candida* and these will be examined.

**Fermentation of Sugars by Candida:**— An interesting group of yeasts reported to assimilate, but not ferment, disaccharides was investigated by KLUYVER and CUSTERS. Included in their study was a strain of *Candida parakrusei,* reported to assimilate both maltose and sucrose, but ferment neither, and three strains of *Candida albicans* reported not to ferment sucrose but able to assimilate it. As seen in Table 1, there is no question about the ability of any of these strains to utilize maltose or sucrose, though *C. parakrusei* is a poor user of maltose, particularly when compared with the values of *Saccharomyces cerevisiae.*

Although four different methods of detection were employed, no evolution of $CO_2$ by *C. parakrusei* from maltose could be detected. Quantitative measurements were made in the van Iterson-Kluyver apparatus (*see* KLEIN, 1933) and by the Warburg manometric technique. With a sucrose substrate, both species and all four strains showed a slight but unmistakable evolution of $CO_2$ anaerobically (*see* Fig. 1). It will be observed that with both species there is a definite production of $CO_2$ anaerobically in the absence of added substrate; this is in contrast to the well-confirmed findings of STIER and STANNARD (1936) for *S. cerevisiae* where no gas or heat exchange has been observed anaerobically in the absence of added substrate (this point will be considered later in this chapter). Additions of sucrose about doubled the rate of $CO_2$ production in all strains.

Although agreeing with MARTIN *et al.* on the lack of gas production by *C. parakrusei* from maltose, the finding of gas production by three strains of *C. albicans* from sucrose is at variance. It is to be emphasized that the amount of gas produced is small, could escape detection qualitatively, and that the experimental conditions were much different from those employed by MARTIN *et al.,* so there is, for all practical purposes, no conflict of statement here. The quantitative detection of $CO_2$ production from sucrose by *C. albicans* has been confirmed by VAN NIEL and COHEN (1942).

**Aerobic Fermentation by Candida:**— An aerobic fermentation of both maltose and sucrose was found by KLUYVER and CUSTERS to occur with *C. parakrusei,* as Figure 2 shows. This fermentation occurring in the presence of oxygen was greater than the anaerobic fermentation of sucrose. The presence of hydrolases for sucrose and maltose is thus demonstrated; these enzymes must be partially or completely inactivated anaerobically. It was suggested by KLUYVER and CUSTERS that possibly the increased state of reduction in the in-

TABLE 1.—*Utilization of disaccharides by species of Candida in sugar-yeast-water media* (KLUYVER and CUSTERS, 1940):—

| ORGANISM | *Incubated 16 days at 30° C.* Sugar consumed, mg. | *Incubated 16 days at 30° C.* Sugar consumed in % of initial amount | *Incubated 35 days at 30° C.* Sugar consumed, mg. | *Incubated 35 days at 30° C.* Sugar consumed in % of initial amount |
|---|---|---|---|---|
| | Maltose | (1,443 mg./flask = 2.1% | | sugar) |
| *C. parakrusei* | 112 | 7.7 | 287 | 19.9 |
| *Saccharomyces cerevisiae* | 1,437 | 99.6 | 1,440 | 99.8 |
| | Sucrose | (1,458 mg./flask = 2.1% | | sugar) |
| *C. parakrusei* | 663 | 45.5 | 695 | 47.6 |
| *C. albicans* (*No. 493*) | 1,430 | 98.1 | 1,449 | 99.4 |
| *C. albicans* (*Levy*) | 582 | 39.9 | .... | ... |
| *C. albicans* (*No. 417a*) | 1,279 | 87.7 | .... | ... |
| *S. cerevisiae* | 1,455 | 99 8 | .... | .. |

terior of the cell due to the withdrawal of oxygen under completely anaerobic conditions might cause an inactivation of the respective hydrolases. This situation, wherein a substrate can be fermented only in the presence of oxygen, seems to have something in common with the observations of STIER and STANNARD on the inability of *S. cerevisiae* to utilize carbohydrate reserves in the absence of oxygen. In

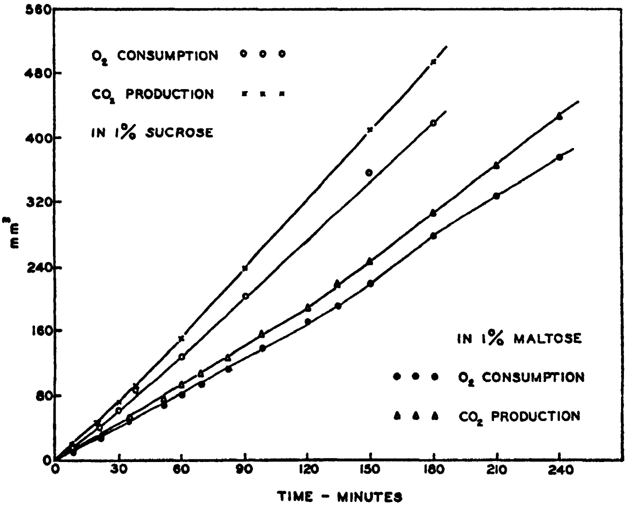


FIGURE 2.—Aerobic metabolism of sucrose and maltose by *Candida parakrusei*. The production of $CO_2$ in excess of $O_2$ consumption is real and interpretable as evidence for an aerobic fermentation of the respective substrates. (KLUYVER and CUSTERS, 1940.)

each case the substrate and enzymes appear to be within the cell but unable to form an activated complex; in the instance with *S. cerevisiae,* spatial relationships appear to be important, as will be discussed later.

Probably the occurrence of an aerobic fermentation with *C. parakrusei* is responsible for the qualitative observations of acid (and slight gas) production from sucrose and galactose referred to earlier.

KLUYVER and CUSTERS attempted to exaggerate the negative Pasteur effect by adding cysteine to suspensions of *C. parakrusei* fermenting aerobically but found no increase in $CO_2$ production. The cysteine was, however, readily converted to $H_2S$ (sulfur competing with oxygen as a hydrogen acceptor) though respiration was not inhibited (the studies of RUNNSTRÖM and BRANDT, 1941, on the effects of cysteine and glutathione on the Pasteur effect are of interest in this connection).

**Oxidation of Substrates by Candida:**— It may be instructive to compare findings on the oxidation of substrates by *Candida albicans* with our information on the process as observed in other yeasts (nonpathogenic) and in other pathogenic fungi. *Candida parakrusei* was found by KLUYVER and CUSTERS to oxidize maltose readily (at about half the rate for glucose) as shown in Figure 2. This, then, is an organism that will not ferment maltose but which can assimilate and respire it readily. Both *C. parakrusei* and the three strains of *C. albicans* were found to oxidize sucrose readily at slightly less than the rate for glucose (*see* Fig. 2). In all of these experiments the cells were obtained from growths on malt agar plates, washed, and suspended in phosphate buffer at *p*H 4.5; the cells were not reported to be starved. There is no question, from the data of KLUYVER and CUSTERS, about the increase in rate over the endogenous rate of oxygen consumption prompted by the addition of glucose, sucrose, or maltose.

Using a single strain of *C. albicans,* VAN NIEL and COHEN (1942) found that suspensions prepared directly from yeast-agar plates showed a high endogenous rate of oxygen consumption (rate in absence of external substrate) that declined rather rapidly with time. In contrast to the findings of KLUYVER and CUSTERS, the addition of oxidizable sugars to such suspensions brought about little increase in the rate of oxygen consumption. With many other yeasts (*e.g.* species of *Saccharomyces, Zygosaccharomyces, Saccharomycodes,* and *Schizosaccharomyces*) the addition of oxidizable substrates to suspensions prepared directly from nutrient media is generally followed by a rapid rise in oxygen consumption to what is frequently termed the exogenous rate. Among the yeasts with these pronounced dissimilative abilities is the familiar bakers' and brewers' yeast, *Saccharomyces cerevisiae.*

Suspensions of *C. albicans* could be prepared by VAN NIEL and COHEN which did oxidize added substrate through the simple procedure of placing growths from nutrient media into sterile tap water or sterile iso-osmotic buffer and aerating by shaking in a shallow layer or by bubbling sterile air or oxygen through the liquid. After several hours, during which time the cells had been respiring at their endogenous or autorespiration rate, intracellular reserve stores of oxidizable material were depleted. Addition of oxidizable substrates to such starved preparations was followed by a rapid rise in oxygen consumption to a high level. However, the quantity of oxygen theoretically necessary for complete oxidation of the added substrate was rarely consumed (Table 2). Rather the oxidation process ceased with only one third of the glucose or sucrose oxidized, the remainder of the sugar being presumably converted to an assimilation product of the general formula ($CH_2O$) as suggested in statement (1), Table 3.

TABLE 2. — *Oxidation of various substrates by C. albicans* (data from VAN NIEL and COHEN, 1942): —

| SUBSTRATE | *Amount added, µ mols* | $O_2$ *uptake µl* | $CO_2$ *produced µl* | *Theoretical for complete oxidation* (*µl* $O_2$ or $CO_2$) | | *Per cent of substrate oxidized based on* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $O_2$ | $CO_2$ | |
| Glucose ....... | 5 | 210 | 226 | 672 | | 31 | 33 | ⅓ |
| | 5 | 228 | 235 | 672 | | 34 | 35 | |
| | 5 | 218 | 224 | 672 | | 32 | 33 | |
| Sucrose ....... | 2.5 | 200 | 210 | 672 | | 30 | 31 | ⅓ |
| | 2.5 | 224 | 230 | 672 | | 33 | 34 | |
| | 2.5 | 218 | 226 | 672 | | 32 | 33 | |
| Acetate ....... | 5 | 110 | 112 | 224 | | 49 | 50 | ½ |
| | 5 | 113 | 115 | 224 | | 50 | 51 | |
| | 10 | 224 | 225 | 448 | | 50 | 50 | |
| | | | | *µl* $O_2$ | *µl* $CO_2$ | | | |
| Ethanol ....... | 10 | 430 | 225 | 672 | 448 | 64 | 50 | ½ |
| | 10 | 400 | 210 | 672 | 448 | 60 | 47 | |
| | 10 | 444 | 230 | 672 | 448 | 66 | 51 | |
| Pyruvate ..... | 10 | 338 | 447 | 560 | 672 | 60 | 60 | ⅔ |

TABLE 3. — *Overall statements suggested for the oxidation of various substrates by C. albicans in the absence of nitrogenous substrates* (VAN NIEL and COHEN, 1942): —

(1) Glucose: $C_6H_{12}O_6 + 2\ O_2 \rightarrow 2\ CO_2 + 2\ H_2O + 4(CH_2O)$
(2) Ethanol: $C_2H_5OH + 2\ O_2 \rightarrow CO_2 + 2\ H_2O + (CH_2O)$
(3) Acetate: $CH_3COOH + O_2 \rightarrow CO_2 + H_2O + (CH_2O)$
(4) Pyruvate: $CH_3COCOOH + 1.5\ O_2 \rightarrow 2\ CO_2 + H_2O + (CH_2O)$

**Assimilatory Processes:**— BARKER (1936) demonstrated that with suspensions of non-proliferating cells of the colorless alga, *Prothotheca Zopfii,* only part of the disappearing non-nitrogenous substrate could be accounted for by oxidation since a large fraction of it had been converted into cellular material. Since this demonstration it has been established by several investigators for a variety of organisms that the phenomenon of assimilation can occur aerobically and anaerobically with "resting" cells.

TABLE 4.—*Comparison of rates of oxygen consumption by different yeasts in the presence and absence of substrate:*—

| | *Endogenous* | | *Exogenous (glucose)* | | |
|---|---|---|---|---|---|
| ORGANISM | Temp. C.° | $Q_{O_2}$ * | Temp. C.° | $Q_{O_2}$ * | Reference |
| *Saccharomyces cerevisiae* ... | 25 | 11.8 | 25 | 33.7 | 58 |
| *S. cerevisiae* (biotin deficient cultures) ............... | | | 30 | 2.8-9.3 | 73 |
| (biotin intermediate) ..... | | | 30 | 14.0 | 73 |
| (biotin rich) ............ | | | 30 | 70-75 | 73 |
| *S. cerevisiae (No. 812)* .... | 30.2 | 28.1 ± 1.1 | 30.2 | 61.0 ± 3.5 | 56 |
| *S. carlsbergensis* ........... | 30.2 | 31.0 ± 1.8 | 30.2 | 82.3 ± 4.9 | 56 |
| *Schizosaccharomyces octosporus* .................. | 30.2 | 21.0 ± 0.9 | 30.2 | 90.2 ± 1.8 | 56 |
| *Schiz. pombe* ............. | 30.2 | 17.9 ± 1.2 | 30.2 | 36.4 ± 1.8 | 56 |
| *Saccharomycodes Ludwigii* . | 30.2 | 38.0 ± 2.3 | 30.2 | 144 ± 6.4 | 56 |
| *Zygosaccharomyces acidifaciens:* | | | | | |
| (24 hr. culture) ........ | 28 | 23 | 28 | 60 | 43 |
| (48 hr.) .............. | 28 | 10 | 28 | 35 | 43 |
| (72 hr.) .............. | 28 | 9 | 28 | 35.5 | 43 |
| (anaerobically grown, 72 hr.) ................ | 28 | 13 | 28 | 17 | 43 |
| *Candida albicans* ........... | 29.6 | 5 | 29.6 | 40 | † |
| *Brettanomyces Claussenii:* | | | | | |
| (64 hr. culture) ....... | | | | 37.8 | 12 |
| (168 hr.) .............. | | | | 27.5 | 12 |
| (anaerobically grown, 144 hr.) ................ | | | | 10.1 | 12 |

* $mm^3O_2$ consumed/mg. dry wt./hr.

† NICKERSON and EDWARDS previously unpublished (65 hr. culture, "starved" by aeration).

It should be noted that BARKER, VAN NIEL, and other authors have referred to the assimilated product as ($CH_2O$) but SCHADE and THIMANN (1940) showed with *Leptomitus* that nitrogen is also involved and that the assimilation product in this organism has about the formula of alanine (incidentally this has the same state of oxidation as a carbohydrate). Thus it seems clear that the "equations" such as (1) may be no more than a form of over-all statement rather than a true equation for a definite reaction. In fact, the quantity expressed as ($CH_2O$) may not even be inside the cell, for PICKETT and CLIFTON (1943) have shown that with suspensions of *S. cerevisiae* in a glucose solution half the material expressed as assimilated ($CH_2O$) from manometric studies in reality occurred free in the medium as soluble end products with an aggregate composition of $(CH_2O)$. Even in the absence of a nitrogen source the product actually assimilated in the cells may be other than a carbohydrate, for STIER, NEWTON and SPRINCE (1939) have shown starved suspensions of bakers' yeast can synthesize both fat and carbohydrate aerobically from glucose. While it is possible in this instance that the carbohydrate was transformed into fat there seems no doubt from the work of DE BOER (1928) but that *Phycomyces Blakesleeanus* assimilates and metabolizes fat preferentially.

**Inhibition of Assimilation Processes:**— It was found by CLIFTON (1937) that the addition of 2, 4-dinitrophenol (DNP) to respiring preparations of microorganisms increased the consumption of oxygen to the theoretical value for complete oxidation of the substrate present. It was believed at first that this represented an inhibition of the assimilation process, but, in at least one instance, such interpretation may not be valid. BURRIS and WILSON (1942) found with the nitrogen-fixing root nodule bacterium, *Rhizobia trifolii,* 205, that the addition of DNP following the levelling off of the initial high rate of $O_2$ consumption resulted in a rapid rise in the rate of oxygen uptake, approaching the initial rate. This return in rate persisted until the theoretical value for complete oxidation of the added substrate was reached. In this case it has been considered (BURRIS and WILSON, 1945) that DNP brought about a rapid oxidation of assimilated materials. If such were true, it is hard to understand why the velocity of the catabolic reaction should decrease when the "assimilated" material from the substrate added to the resting cells was consumed, and why the process of rapid oxidation should not continue to feed on materials previously assimilated by the cells during growth unless there are decided differences in the two assimilated materials or in their utilization. The stage at which DNP may reverse the assimilation process in *Rhizobia* (or in any other organism) has not been demonstrated, but it would seem clear that the material oxidized following the addition of DNP may not have the same "status" in the cell as previously assimilated materials.[1] There appears to be some parallel here with the findings of Barker (1936) that *Prototheca Zopfii* continues to oxidize, though at a reduced rate, the material immediately assimilated into the cell from a glycerol substrate. The oxidation of this assimilated, but not "fixed" substance declined in rate until the endogenous rate once again was reached.

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[1] An apparently more complicated situation is found with the luminous bacterium, *Achromobacter Fischeri,* as reported by MCELROY (1944). Assimilation (and luminescence) inhibitors stimulated a small additional oxygen uptake which appeared to be at the expense of material assimilated during growth and respired via the endogenous system (which is not suppressed on the addition of substrate). There were indications that chloral hydrate also stimulated the oxidation of primary assimilation products, if added before the disappearance of glucose, but not if added afterwards. Barbiturates, chloral hydrate, chloretone, and DNP did not increase the rate or extent of oxidation of glucose (only 17% of the theoretical for complete oxidation). The first two inhibitors had little effect if added after glucose had disappeared, but chloretone caused an immediate rise in oxygen uptake at 19° C., but not at 25° C., attributable to the oxidation of products of primary assimilation.

Other substances than DNP are known that interfere with the assimilation process; some of these are arranged in Table 5. It appears that a variety of substances are able to inhibit assimilation and concomitantly stimulate some catabolic process.

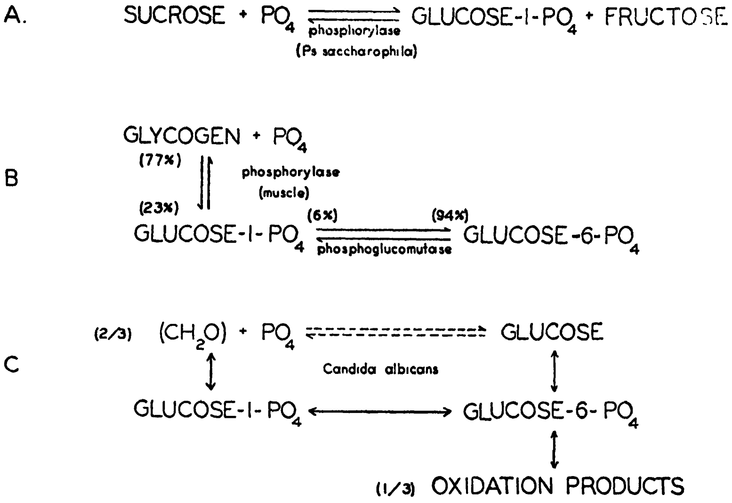

Figure 3. — *Upper two reactions are syntheses achieved with in vitro preparations that may be intimately associated with in vivo assimilation processes.*—(A) achieved with *Pseudomonas saccharophila* (Doudoroff *et al.*, 1944). (B) shown with muscle preparations (*see* Cori, 1941); the equilibria values attained indicate approximately a 5% conversion of glucose to glycogen. Contrast with the *in vivo* findings for assimilation of ($CH_2O$) in *C. albicans* of ⅔ of the carbon from glucose. Broken arrows indicate the apparent reaction in (C) and solid double-headed arrows a possible path, not proved.

Runnström and Brandt (1941) found that glutathione inhibited assimilation from glucose by bakers' yeast, while stimulating aerobic glycolysis. The respective relations of the three processes (oxidation: assimilation:aerobic glycolysis) was 4:6:0 which changed to 4:0:6 following the addition of glutathione. This negative Pasteur effect was interpreted by the authors as a diversion of glucose phosphate to fermentative decomposition away from pathways leading to glycogen synthesis. It is of interest that respiration is not stimulated in this example; it might be that the respiratory enzyme systems were working at saturation prior to the addition of glutathione and the excess "pressure" (by analogy with Mass Action) of glucose-phosphate, developed by the blocking of assimilation pathways, is shunted via aerobic glycolysis paths. CORI (1941) had found that reducing agents, such as glutathione, markedly shift the equilibria of the reactions shown in Figure 3 in favor of the formation of glucose-6-phosphate. This is away from the synthesis of glycogen, and the findings of RUNNSTRÖM and BRANDT may be explainable in this manner. Along these lines KLUYVER and CUSTERS were able to inhibit the Pasteur effect in *Torulopsis dattila* with 6 mg. per cent (M/2000) cysteine (to which component glutathione owes its reducing activity) securing a pronounced aerobic fermentation of maltose which *T. dattila* oxidizes and assimilates readily, but which it does not ferment anaerobically at all. Respiration was inhibited in these experiments with cysteine.

TABLE 5.—*Comparison of effects of substances inhibiting assimilation:*—

| COMPOUND | *Concentration* | *Organism* | *Process stimulated* | *Reference* |
|---|---|---|---|---|
| | | *Aerobically* | | |
| Dinitrophenol . | M/8000 | Ps. calco-acetica | Respiration | 9 |
| Dinitrophenol . | M/1000 | S. cerevisiae | Aerobic fermentation | 49 |
| Sodium azide .. | M/600 | Ps. calco-acetica | Respiration | 9 |
| Iodoacetate ... | M/20000 | Ps. calco-acetica | Respiration | 9 |
| Glutathione ... | M/140 | S. cerevisiae | Aerobic fermentation | 51 |
| Gramicidin .... | ........ | S. cerevisiae | Respiration* | 32 |
| | | *Anaerobically* | | |
| Sodium azide .. | M/1000 | S. cerevisiae | Fermentation | 71 |

* Phosphate uptake from the medium also inhibited.

**Problems of Spatial Orientation of Substrates and Enzymes in Cells:**— One of the most striking examples of the governing influence of spatial arrangements on metabolic processes seems to be the lack of detectable gas exchange[2] (STIER and STANNARD, 1936) or heat production (WINZLER and BAUMBERGER, 1938) in the cells of many yeasts under anaerobic conditions in the absence of external substrate. Destruction of the cellular organization, as by grinding of the cells, is rapidly followed by a fermentation of substrate stored within the cell (STIER and STANNARD). Aerobically, in the absence of added substrate, the dissimilation of stored reserves by yeasts appears to proceed only by oxidative paths. This was shown for *S. cerevisiae* by STIER and STANNARD and confirmed by SPIEGELMAN and

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[2] CHUDIAKOW (1894), using a sensitive Pettenkofer $CO_2$-absorption assembly, had shown there is no carbon dioxide production by yeast in a hydrogen atmosphere in the absence of fermentable substrates. In one experiment with 3% quinic acid, 1% peptone, and mineral salts, there was an average carbon dioxide production of 11.7 mg./hr. at 35° C. with a 3 liter/hr. ventilation with air. Under identical conditions with ventilation by hydrogen, only traces (< 0.1 mg.) of carbon dioxide were produced.

NOZAWA (1945) for *Saccharomyces, Schizosaccharomyces,* and *Saccharomycodes;* comparative data are shown in Table 6. It seems quite clear that stored reserves in most of the yeasts studied cannot be metabolized (in the absence of added substrate) either aerobically or anaerobically by fermentative paths in the intact cell; the fact that destruction of the cell organization permits fermentation to occur would tend to support the view that stored reserves are separated spatially from the fermentation systems. As a corollary, the enzymes for endogenous respiration must be associated with reserve stores and similarly removed from the fermentative enzyme and exogenous respiration enzyme systems.

TABLE 6. — ***Comparison of anaerobic gas exchange by yeasts in the absence of added substrate*** (each species ferments glucose at least) : —

| ORGANISM | *Temp.* C.° | *p*H | *Gas exchange* | $Q^{CO_2}_{N_2}$ | *Reference* |
|---|---|---|---|---|---|
| *Saccharomyces cerevisiae (No. 4360)* | 25 | 4.5 | negative | 1.4 $mm^3CO_2/hr.$* | 58 |
| *S. cerevisiae (LK2G12)* | 30.2 | 4.5 | negative | 0.2 ± 0.5 | 56 |
| *S. cerevisiae (No. 812)* | 30.2 | 4.5 | negative | 0.1 ± 0.1 | 56 |
| *Saccharomyces carlsbergensis* | 30.2 | 4.5 | negative | 0.4 ± 0.1 | 56 |
| *Saccharomyces italicus* | 30 | 4.5 | negative | *n. m.* | 35 |
| *Saccharomycodes Ludwigii* | 30.2 | 4.5 | negative | 0.9 ± 0.2 | 56 |
| *Brettanomyces anomalus* | 30 | 4.5 | negative | *n. m.* | 35 |
| *Torulopsis dattila* | 30 | 4.5 | negative | *n. d.* | 35 |
| *Schizosaccharomyces octosporus* | 30 | 4.5 | doubtful | 3 $mm^3CO_2/hr.$* | 35 |
| *Schiz. octosporus* | 30.2 | 4.5 | negative | 0.1 ± 0.06 | 56 |
| *Schiz. pombe* | 30.2 | 4.5 | negative | 0.4 ± 0.1 | 56 |
| *Candida albicans (No. 493)* | 30 | 4.5 | positive | 20 $mm^3CO_2/hr.$* | 35 |
| *Candida parakrusei* | 30 | 4.5 | positive | 15 $mm^3CO_2/hr.$* | 35 |
| *Torula sp. (Claussen)* | 30 | 4.5 | positive | 35 $mm^3CO_2/hr.$* | 35 |

* Figures for dry wt. not given.
*n. m.*, not measurable; *n. d.*, not detectable.

Evidence from another source supports the view of spatial separation of the endogenous and exogenous systems. SELZER and BAUMBERGER (1942) showed that the endogenous respiration in *S. cerevisiae* is not influenced by metallic mercury in the suspending medium for the cells, but the exogenous respiration system is markedly inhibited. They suggested that the sulfhydryl groups (specifically inhibited by mercury) of the endogenous respiration systems are located within the interior of the cell, while the thiol concerned in the exogenous respiration system is located in or near the cell wall.

An interesting aspect of the relations, in the absence of substrate, of yeasts to oxygen is that while aeration reduces the endogenous oxygen consumption markedly in a few hours, suspensions can be stored anaerobically for at least 72 hours in buffer alone without decline in the endogenous rate on return to oxygen. This was shown by NICKERSON and CARROLL (1943) for *Zygosaccharomyces* and confirmed by SPIEGELMAN and NOZAWA for *Saccharomyces, Schizosaccharomyces,* and *Saccharomycodes.* The question was raised by both authors regarding the energy requirements of these cells anaerobically. It has frequently been assumed that cells utilize energy to maintain their intact condition with their components in some sort of order. But here are examples of cells that apparently carry out little or no energy exchange and yet exist without impairment to a complicated internal structure that immediately responds (unlike spores) to the addition of oxygen or substrates. A process operating with yeast cells stored aerobically is the loss (or secretion) of riboflavin or thiamin into the buffer, with consequent impairment of cellular function which can be overcome by subsequent replacement of the lost substance. Such a loss does not seem to occur anaerobically to any appreciable extent and appears to be one process distinguishing the two methods of storage. It is curious that in at least one instance the invigoration of resting (and resistant) structures is conditioned by the external supply of diffusible substances implicated, as are thiamin and riboflavin, in cellular respiration processes. This situation occurs in the stimulation of excystment in *Colpoda* (HAAGEN-SMIT and THIMANN, 1938) by members of the four carbon dicarboxylic acid series.

Three yeasts investigated by KLUYVER and CUSTERS apparently can carry out an anaerobic gas exchange in the absence of added substrate, as mentioned in Table 6 and shown in Figure 1. The anaerobic conditions were carefully produced by passing a nitrogen-carbon dioxide mixture through heated copper coils; the aerobically grown cells had been washed three times and resuspended in phosphate buffer. The conditions were the same as those under which other yeasts did not produce gas. VAN NIEL and COHEN also found carbon dioxide (and a very slight amount of acid) produced by *C. albicans* anaerobically in the absence of substrate. It seems clear that the situation with *Saccharomyces* is not universally distributed among yeasts.

**Physiological Age of Cells vs. Assimilation:**— Assimilation of substrates by cells seems to be influenced by age of the cells and their culture history. NICKERSON and CARROLL (1943) pointed to the effect of age and culture history of cells on the rates of oxygen consumption by suspensions of the yeast *Zygosaccharomyces acidifaciens* showing cultures 48 hours old had but ½ the $Q_{O_2}$ of 24 hour cultures (*see* Table 4). BERNSTEIN (1944) found suspensions of young cells of *Pseudomonas saccharophila* oxidize ½ the molecule and assimilate the other half from succinic, fumaric, and *l*-malic acids. With older cells (defined by the *p*H to which cell growth had changed the H+ concentration of the medium) the same fraction of the substrate was assimilated but two distinct steps occurred in the oxidation; pyruvic acid accumulated as an extracellular product in the first phase, disappearing in the second phase. VAN NIEL and ANDERSON (1941), confirming and amplifying the demonstration by WINZLER and BAUMBERGER (1938) that assimilation of substrate may occur anaerobically as well as aerobically, found 30 percent of the substrate assimilated by relatively young cells (2-5 days) of *Saccharomyces cerevisiae,* but only 7-8 percent assimilated by old cells (28 days).

The process of producing "starved" preparations of microorganisms may have effects on the cells other than mere depletion of oxidizable reserves. STIER (1939) showed that after removal of cells from their nutrient medium, aeration of *Saccharomyces cerevisiae* in a phosphate buffer resulted in the production of suspensions exhibiting a lowered aerobic metabolism of dextrose when the suspensions were supplied with that sugar. GIESE (1942) found that the endogenous respiration of *S. cerevisiae* exhibits a decline with increase in age of the cells as well as with aeration after removal of the cells from their culture medium. STIER and MACINTYRE (1942) have shown a rapid loss of riboflavin from cells of *S. cerevisiae* into the storage buffer during aeration of the yeast suspension. VAN NIEL and COHEN found that aerobically grown 2-3 day old cells of *Candida albicans,* after 20 hours' aeration of a suspension in tap water, are apparently deficient in cocarboxylase, as judged by the rate of oxidation of pyruvic acid by such cells. The rate of pyruvate oxidation could be doubled by the addition of thiamin to the starved cells.

**Discussion:**— The process of liberating energy for cellular activities through the uptake of molecular oxygen by the cell in the course of oxidizing its particular substrates furnishes a wealth of sites for chemotherapeutic attack on the cell if it be one whose continued existence is deemed undesirable. While the basic concepts of the mechanisms of hydrogen activation, hydrogen transport, oxygen activation, oxygen transport, and phosphorylation that have been elucidated appear in the main to hold true for cells generally, the catalytic mechanisms involved in cellular respiration are manifold, and multiple pathways of oxidation are known to exist. While even related species may carry out apparently identical oxidations, it is known that in some cases the mechanisms of oxidation may vary in important aspects, as BARRON (1942) has pointed out.

Throughout this chapter the attempt has been made to compare our knowledge of the oxidative and fermentative processes carried out by pathogenic fungi with the much more extensive information available for non-pathogenic forms. From this comparative point of view we may be able to select favorable avenues for experimentation with the pathogenic fungi. To establish a basis for comparison with nonpathogenic forms it has been necessary in some instances to devote much of a section to papers of purely theoretical nature; no apology is felt necessary for this.

### *II. Blastomyces*

A most interesting group of organisms for investigation of respiratory and fermentative activity would seem to be those fungi causing systemic mycoses. These serious pathogens exist in man in a single cell, yeast-like state, but in culture may be maintained either in a yeast-like or in a filamentous condition (the role of temperature in this duality is discussed in Chapter 9). Included among these fungi are *Blastomyces dermatitidis* and *Histoplasma capsulatum*. So far as could be learned, only one study has been made of the gaseous metabolism of a fungus causing systemic disease; indeed, little information is available on any aspect of their physiology.

TABLE 7.—*Respiratory quotient and fraction consumed of oxygen necessary for complete oxidation of a substrate by B. dermatitidis ("A" calculated on basis of persistence of endogenous rate, and "B" assuming suppression of endogenous rate on addition of substrate; temp. 37° C.; pH 6.7; data recalculated from* BERNHEIM, *1942)*:—

| SUBSTRATE | *Fraction consumed of $O_2$ necessary for complete oxidation* *"A"* | *"B"* | *Observed R.Q.* | *Theoretical R.Q. for oxidation as in "B"* |
|---|---|---|---|---|
| Endogenous | ... | ... | 0.80 | ... |
| Glucose | 1/6 | 1/3 | 0.96 | 1.0 |
| Pyruvate | 1/5 | 3/5 | 1.53 | 1.33 |
| Acetate | 1/4 | 1/2 | ... | 1.0 |

In the first study of the oxidative metabolism of an organism causing systemic fungus disease, BERNHEIM (1942) investigated *Blastomyces dermatitidis*. Unstarved preparations of the *yeast* form of this organism grown on Kelly's agar medium oxidized added glucose, mannose, lactate, and pyruvate in M/20 phosphate buffer at *p*H 6.7, or at *p*H 7.8. The *mycelial* form of *B. dermatitidis* was reported to exhibit a lower rate of oxygen uptake and to oxidize added substrates more slowly. Sugars are not fermented by either growth-form of *B. dermatitidis*. Glucose and mannose were oxidized at the same rate and took up 2 mols of oxygen per mol, provided the high endogenous rate is considered suppressed (*see* Table 7). Of five sugars tested, arabinose, rhamnose, and, very strangely, fructose, were not oxidized. The endogenous rate of oxygen uptake was approximately one half of the rate with added glucose.

Aliphatic aldehydes were oxidized, but aromatic aldehydes inhibited the endogenous respiration; anisaldehyde prompted a 15-25% inhibition of the endogenous rate. Curiously, this substance was without effect on the oxidation of added glucose but inhibited the oxidation of pyruvate and of lactate; the effect of anisaldehyde and two other inhibitors, and the oxidation of different substrates is compared in Table 8.

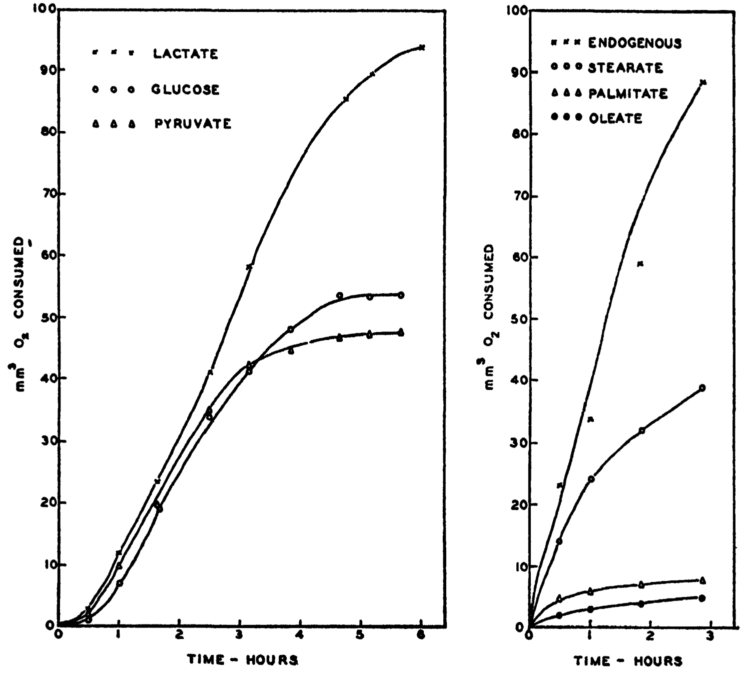

FIGURE 4. — Oxygen consumption by *Blastomyces dermatitidis* with different substrates (graph on left) and effect of higher fatty acids on endogenous rate (right). Endogenous oxygen consumption subtracted on left; incomplete oxidation of substrates at breaks in curves. (BERNHEIM, 1942.)

As given by BERNHEIM, the oxidation of all substrates tested with *B. dermatitidis* is most incomplete, as a glance at Table 7 shows; unfortunately, he did not present any analyses for these substances in the vessels or analyses on the cells after the completion of a manometric experiment. The curves in Figure 4 would indicate that oxygen consumption due to added substrate had ceased with incomplete oxidation of the substrates. If one were to assume that this is a case of incomplete oxidation accompanied by assimilation, as with *Candida*, then for acetate (one atom of oxygen taken up and one molecule of

$CO_2$ given off per molecule of acetate) a statement of the oxidation would appear to be:

$$CH_3COOH + \frac{1}{2}O_2 \rightarrow CO_2 + H_2O + (\text{—}CH_2\text{—}).$$

This would be an unusual type of assimilation reaction with the carbon residue having the state of oxidation of an unsaturated hydrocarbon. In view of the high lipid content of this organism, as revealed by PECK and HAUSER (1938, 1940; *see* Chapter on Lipids), this statement might conceivably have significance. Much more likely, however, is it that the procedure used by BERNHEIM of subtracting the values for endogenous oxygen consumption from the observed values in the presence of substrate gives values for oxidation that are too low. It is frequently a real problem to decide whether or not one should correct for the endogenous respiration; the discussion by BARKER (1936) of the problem is valuable in this connection. In the present instance, it seems clear from the R. Q. values presented in Table 7 that the endogenous respiration (R. Q. of 0.8 indicative of fatty acid metabolism) is suppressed nearly completely on the addition of substrate since the R. Q. with glucose of 0.96 indicates oxidation of carbohydrate and hardly any (or but little) of a mixed fat-carbohydrate oxidation. Since the rate of endogenous respiration is about half of the rate with added glucose, one would expect an R.Q. of 0.87 if both the endogenous material and the glucose were being oxidized simultaneously. A recalculation based on suppression of the endogenous oxygen consumption gives values for the fraction of substrate oxidized (*see* Table 7) similar to those found for *Candida* and other organisms (*see* Table 2).

TABLE 8.—*Effect of various inhibitors on the oxygen consumption of Blastomyces dermatitidis with different substrates (temp. 37° C.; pH 6.7; data from* BERNHEIM, *1942)*:—

| SUBSTANCE | *Concentration* (mg. %) | *Endogenous respiration* | *Oxygen uptake with substrates* | | |
|---|---|---|---|---|---|
| | | | Glucose | Lactate | Pyruvate |
| Anisaldehyde . | 50 | 15-25% inhibited | no effect | inhibited | inhibited |
| Iodoacetate ... | 50 | 40% inhibited | strongly inhibited | inhibited | stimulated |
| Sodium selenite .... | 10 | 80% inhibition developed slowly | strongly inhibited | ........ | stimulated |
| Sodium oleate ...... | 50 | strongly inhibited | inhibited | inhibited | inhibited |

The higher fatty acids are not oxidized and inhibit oxygen uptake on all substrates tested (*see* Table 8); sodium oleate and palmitate were about equally effective as shown in Figure 4.

When amino acids were added to washed suspensions of *B. dermatitidis,* Bernheim reported they increased oxygen consumption over the control in the absence of substrate, but in no case did the amount of oxygen consumed indicate complete oxidation of any of the amino acids added; rather, the oxygen consumed was only sufficient to permit of an oxidative deamination; but such an occurrence did not seem likely, since the amount of ammonia produced was too low to agree with an oxidative deamination. Analyses for amino-nitrogen gave equivocal results; with *l*-phenylalanine all the amino-nitrogen seemed to remain unchanged in the medium, while with glycine 20%, and with alanine 12% of it disappeared. Yet all three compounds stimulated oxygen consumption. Bernheim interpreted his data to mean that the amino acids are not oxidized nor oxidatively deaminated, yet are able to stimulate the oxidation of some unknown substance in the cell. Such an interpretation is rather unsatisfying and it seems more likely that the amino acids may have been oxidized to substances of a higher oxidation state( such as alanine) without deamination occurring.

The above situation has a striking parallel with that found in *Leptomitus lacteus* by Schade and Thimann (1940) with the amino acid *l*-leucine, which was neither deaminated nor completely oxidized, yet which stimulated a considerable increase in oxygen consumption over the endogenous. Their interpretation was that *l*-leucine was oxidized to a compound having the same oxidation state as alanine which was then directly assimilated, as shown in the following overall equation:

$$C_4H_9CHNH_2COOH + 4\tfrac{1}{2}O_2 \rightarrow (C_3H_9NO_2) + 3\ H_2O + 3\ CO_2.$$

## DERMATOPHYTES

**Introduction.** — With the dermatophytes the problems of handling filamentous fungi arise in making preparations for respiratory studies. By reason of their filamentous structure, these organisms are not so convenient to handle experimentally as are the unicellular organisms; a few references will be made to methods that have been used in work with these forms.

De Boer (1928), in large-scale studies on *Phycomyces Blakesleeanus,* employed Pettenkofer absorption tubes and a titration procedure to measure carbon dioxide production. Oxygen consumption was determined by a modification of the Fernandes (1923) device that utilizes the electrolytic hydrolysis of NaOH to supply oxygen at constant pressure, the oxygen used being calculated from the volume of hydrogen produced during the electrolysis. Schade and Thimann studying a water mold, *Leptomitus lacteus,* utilized the Warburg technique (these methods are well described by Dixon, 1943, and by Umbreit, Burris and Stauffer, 1945) to determine oxygen consumption and carbon dioxide consumption during the respiration of "starved" mycelial preparations of this fungus on a variety of substrates. WOLF and SHOUP (1943), working with four species of the water mold, *Allomyces,* found (as had SCHADE and THIMANN) that it was necessary to employ starved suspensions of the fungi to obtain rates in excess of the control amounts when substrates were added to respiring preparations in plain buffer. KLUYVER and PERQUIN (1933) cultivated molds in flasks placed in a rotating shaking machine for the preparation of homogeneous suspensions of compact mycelia for use in respiration studies. NICKERSON and CHADWICK (1946) employed a volumetric microrespirometer (*see* SCHOLANDER and EDWARDS, 1942) to study the oxygen consumption of three species of dermatophytes; the effects of alterations in environmental conditions upon oxygen uptake of these organisms were studied using vigorous, unstarved mycelia.

**Oxidation of Substrates:**— The rates of oxygen consumption have been studied for several isolates of three species of dermatophytes by NICKERSON and CHADWICK. All of the strains examined were found to possess sufficient intracellular reserve stores of oxidizable materials to maintain a fairly constant rate of oxygen uptake over a period of several hours. These preparations were unstarved. The fungus preparations studied were placed in buffer solutions without an external source of substrate; representative studies on the three species are shown in Figure 5. Many different carbon sources were added to respiring fungus preparations to learn which, if any, were suitable substrates for oxidation by the fungi. At hydrogen ion concentrations on the acid side of neutrality, no compound added promoted any increase in the rate of oxygen uptake over the rate in the absence of the compound. At alkaline *p*H, M/20 dextrose added to *T. gypseum* and M/30 glycerol added to *T. rubrum* were followed by increases of 43 and 36 percent respectively in the rates of oxygen consumption. The rates of oxygen consumption by these organisms in the absence of added substrate are shown for the different species in Table 9 and compared with rates of oxygen consumption for other fungi in Table 10. These relatively slow rates of oxygen consumption, coupled with the non-oxidation of added carbon sources, indicate that these organisms are equipped to oxidize some assimilation product, to

TABLE 9.— $Q_{O_2}$ *of different isolates of three species of dermatophytes* (45): —

| | | $Q_{O_2}$ = *mm³O₂ consumed/mg. dry wt./hr.* | | | |
|---|---|---|---|---|---|
| SPECIES | *No. of expts.* | Low | High | Mean | Sigma |
| *E. floccosum* ................ | 10 | 1.08 | 3.67 | 2.01 | 0.84 |
| *T. gypseum* ................ | 14 | 0.35 | 2.85 | 1.71 | 0.74 |
| *T. rubrum* .................. | 26 | 0.42 | 1.44 | 0.82 | 0.32 |

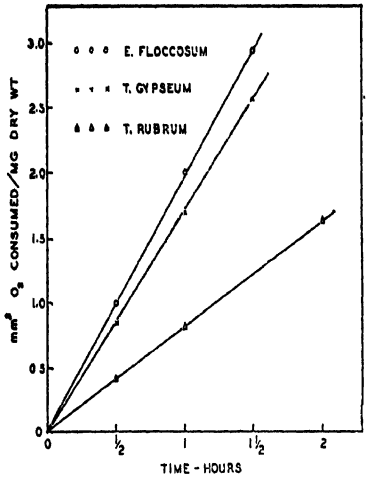

Figure 5. — Representative experiments on oxygen consumption by unstarved preparations of mycelia of three species of dermatophytes. No substrate added; rates were found to remain constant for as long as ten hours. (Previously unpublished figure.)

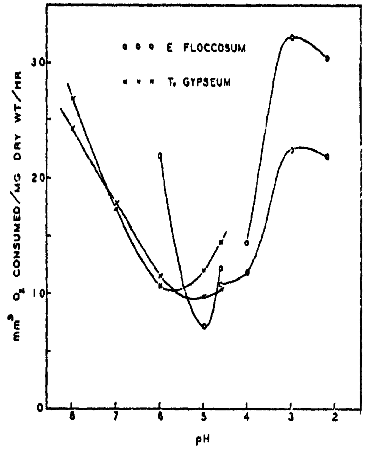

Figure 6. — Variation in the $Q_{O_2}$ of *Epidermophyton floccosum* and *Trichophyton gypseum* with *p*H in McIlvaine's citrate-phosphate buffer. Each point represents rate, which was constant, determined for a period of one or more hours at 28° C. in an oxygen atmosphere. (Nickerson and Chadwick, 1946.)

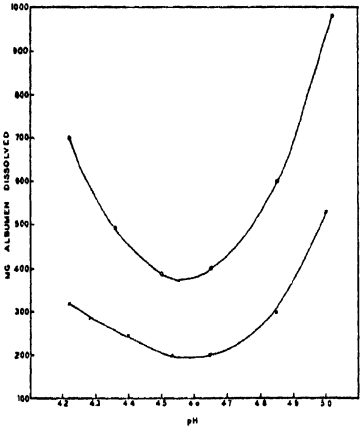

Figure 7. — Effect of *p*H on the "solubility" of crystalline egg albumen in ammonium sulfate solutions (circles, 25.947 g. $(NH_4)_2SO_4$/100 g. $H_2O$; crosses, 27.12 g./100 g.) Ordinate in mg. egg albumen hydrate dissolved per 100 g. water in liquor. Compare with Fig. 6. (Gortner, 1938.)

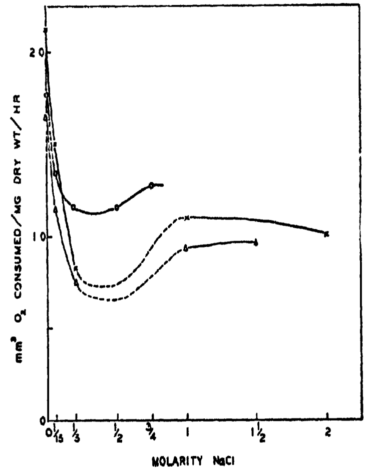

Figure 8. — Variation in the $Q_{O_2}$ of *T. gypseum* with concentration of sodium chloride. Zero concentration of NaCl is rate of $O_2$ uptake in $CO_2$-free distilled water; each point represents rate, which was constant, over 1¼ hour period; new rate assumed almost immediately on changing fungus to next concentration; temp. 28° C.; oxygen atmosphere. (Nickerson and Chadwick, 1946.)

which the dextrose and glycerol might have been converted at alkaline levels. GODDARD (1934) found the efficiency of assimilation very high with *Trichophyton mentagrophytes* (*interdigitale*); the organism degraded 0.625 mg. of protein and 1.0 mg. of glucose for each mg. increase in dry wt. for a period of six days. This gives a ratio of weight of fungus produced to weight of substrate consumed of 1:1.625. A common value with *Phycomyces, Mucor, Neurospora,* etc., is 1:4.

TABLE 10.—*Comparison of rates of oxygen consumption by different fungi:*—

| ORGANISM | *Temp. C.°* | $Q_{O_2}$ | *Substrate added* | *Reference* |
|---|---|---|---|---|
| *E. floccosum* .................... | 28 | 2.05 | none | 45 |
| *T. rubrum* ...................... | 28 | 0.8 | none | 45 |
| *T. mentagrophytes* (*gypseum*) .... | 28 | 1.7 | none | 45 |
| *Leptomitus lacteus* ............. | 20 | 20.5-22.1 | *dl*-alanine | 52 |
| *Neurospora:* | | | | |
| Spores, dormant ............... | 25 | 0.25 | none | 26 |
| Spores, germinating ........... | 25 | 19.60 | none | 26 |
| *Neurospora sitophila* (*No. 299*) .. | 25 | 27.4 | sucrose | 23 |
| *Neurospora crassa* (*No. 1633*) .... | 25 | 21.3 | sucrose | 23 |

**Effect of Hydrogen Ion and NaCl Concentrations:**— With two species of dermatophytes, *E. floccosum* and *T. gypseum,* the rates of oxygen uptake vary remarkably with variation in *p*H (NICKERSON and CHADWICK, 1946). From initial levels at *p*H 2 the rate of oxygen uptake decreases to a minimum in rate in the region of *p*H 5.0-5.5, rising to higher levels, comparabe to the initial rates, at *p*H 8. As shown in Figure 6, the curves suggest the behavior of proteins with varying *p*H; the region of minimum rate corresponding to the isolectric point for proteins, or (to mention one characteristic) the region of their minimum solubility (compare with Figure 7). PFEIFFER (1929) in a review on the isoelectric point in cells and tissues pointed out there is a minimum of physiological function at the isoelectric point of plasma proteins. In view of the apparently analogous reactions of the respiratory enzyme system in these organisms to isolated protein systems with changing acidity, it was desirable to investigate other treatments to which protein systems respond characteristically. A study of the effect of increasing concentrations of sodium chloride on oxygen consumption was undertaken.

To respiring preparations of *T. gypseum* in distilled water increasing concentrations of sodium chloride were added. It can be seen from Figure 8 that the rate of oxygen consumption, highest in distilled water, falls sharply on the addition of small amounts of NaCl only to increase with increasing concentrations of the salt. This situation makes an interesting comparison with the well known behavior of globulins in the presence of increasing concentrations of salt, as discussed in detail by GORTNER (1938).

It is rather important to point out that the rates of oxygen consumption at any given level of NaCl or $H^+$ concentration were constant for the entire time the experiment was conducted at that level (always one or more hours). When a given fungus preparation was changed from one environment to another, a new rate of oxygen uptake was initiated almost immediately; the organisms responded sharply to changes in their environmental conditions.

**Evidence for Duality of Cytochrome System:**— An alternative view of the variation in rates of oxygen consumption with changing hydrogen ion concentrations is that two enzyme systems might be involved, one active in the acid region, the other at alkaline levels. However, it is rather more difficult to account for the observations with sodium chloride on this basis; though it is possible that one system might be inhibited with increasing concentrations of salt and a second system activated.

It will be recalled that TATE (1929 *a* and *b*), using acetone-powder preparations (*see* Chapter 10), demonstrated the presence of indophenol oxidase in several species of dermatophytes, including: *T. Megnini, T. Sabouraudi, T. rubrum,* and *T. mentagrophytes.* It is of considerable interest then that in other instances a two-component system has been proposed (for organisms respiring via the cytochrome system) to account for environmentally influenced responses of the cells. These were the studies of TANG and of BOREI and LINDVALL, which will now be considered.

A response in the rate of oxygen uptake, similar to the findings in Figure 6, to varying hydrogen ion concentrations has been noted at least once previously. TANG (1936) found a minimum rate of oxygen uptake for the yeast, *Saccharomyces wanching,* at *p*H 7.2 (*p*H was 8.9 before adding glucose; see note below) in a borate buffer series; he remarked about the resemblance between his curves for oxygen consumption and the characteristic behavior of many colloidal processes with varying $H^+$ concentrations. In contrast to his findings using a borate buffer series, he observed with phthalate and phosphate buffers that his organism exhibited only a slight decline in oxygen uptake towards the extreme acid or alkaline ranges, while with an acetate buffer the rate dropped very sharply with increasing acidity. Later, TANG (1936*a*) found glucose markedly altered the *p*H of borate buffers and that borate affected the optical activity[3] of glucose solutions, possibly through formation of a complex between glucose and borate, so that his measurements probably did not reflect the response of some component of the cytochrome system to *p*H as first believed.

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[3] RAISTRICK and YOUNG (1931) had earlier made use of the optical properties of glucose and mannitol in 6% borax solutions in developing an analysis for mannitol in the presence of glucose.

Recently, Borei and Lindvall (1943) showed the endogenous respiration (rate of oxygen consumption in the absence of added substrate) of *Saccharomyces cerevisiae,* proceeding via the cytochrome (cyanide sensitive) system, to consist of two fractions. With increasing acidity (*p*H 6.5 to *p*H 4), the rate of one fraction remained unchanged while the second increased in rate, possibly as a result of enzyme activation. Winzler (1940) had previously found a somewhat similar situation with a strain of *S. cerevisiae* in which the endogenous respiration was stimulated about 7 fold (approaching the exogenous rate) by a phthalate buffer at *p*H 3.5 (the endogenous $Qo_2 = 44.2$) and *p*H 4 ($Qo_2 = 39.5$), while at *p*H 5 the endogenous $Qo_2$ had the more normal value of 6. A citrate buffer gave a $Qo_2$ of about 6 throughout a range from *p*H 6 to *p*H 3.5. The phthalate buffer had no effect on the exogenous $Qo_2$ when the yeast was metabolizing acetate.

As an exploratory method for determining the number and properties of the respiratory systems that may be distinguishable in cellular preparations, experiments involving alterations in cell environments have considerable value. Exploratory studies by these means, preliminary to enzyme preparation investigations, may be of material aid. Coupled with studies on specific enzyme inhibitors, the environmental-influence studies may present a more exact view of the respiration system complex as it operates within the cell.

**$Q_{O_2}$ of T. rubrum vs. pH:**— Against the sensitivity of the repiratory systems of *E. floccosum* and *T. gypseum* to changes in $H^+$ concentration, the enzyme system of *T. rubrum* controlling oxygen

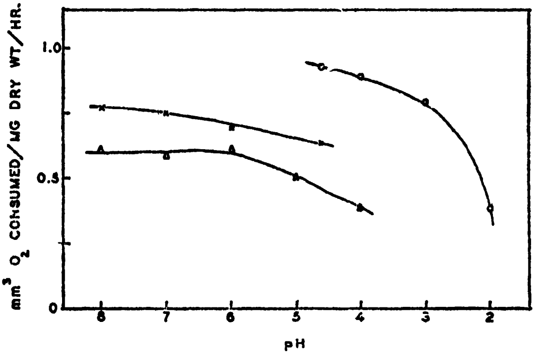


**Figure 9.** — Variation in $Qo_2$ of *T. rubrum* with *p*H. Tendency is mostly a depressing effect with increasing acidity; compare with Fig. 6. Buffers used were citrate-phosphate (triangles), phosphate (crosses), and phthalate (circles) in an oxygen atmosphere at 28° C. Rates constant for 1¼ or more hours. (Previously unpublished figure.)

uptake is in marked contrast (Nickerson and Chadwick, 1946). With phthalate, citrate, and phosphate buffer series (*see* Fig. 9), the rate of oxygen consumption by this organism is seen to change but slightly with variation in *p*H. A slightly decreasing rate of oxygen consumption is shown in Figure 9 for increasing acidities; the $H^+$ concentration in the environment of *T. rubrum* may be said to be without notable effect.

It is the contention of Myrbäck and Vasseur (1943) from work on four species of lactose-fermenting yeasts that enzymes localized at the cell surface may be markedly affected by the *p*H of the cell environment, while enzymes located within the cell are not readily thus influenced. According to this interesting hypothesis, it may be that the respiratory enzyme system of *T. rubrum* is located within the cell, while those of *E. floccosum* and *T. gypseum* are near or in the cell surface, easily influenced by environmental changes (see previous discussion on spatial relations).

In this vein, remembering the lower basal rate of oxygen uptake exhibited by *T. rubrum,* it is of interest to recall that *T. rubrum* is the most stubborn to erradicate of the dermatophytes causing foot infections, as Conant *et al.* (1945) point out. No assertion is made that there is necessarily any connection between the clinical behavior of this organism and the findings on its respiration rate.

Table 11.—*Effect of salts of heavy metals on oxygen consumption by dermatophytes* (45):—

| Compound | *Molar concentration* | *Organism* | *% change in* $Q_{O_2}$ *from control* |
|---|---|---|---|
| $ZnCl_2$ | M/100 | *E. floccosum* | —93 |
| $ZnCl_2$ | M/100 | *E. floccosum* | —92 |
| $ZnCl_2$ | M/1000 | *T. rubrum* | —32 |
| $Zn(NO_3)_2$ | M/1000 | *T. rubrum* | —22 |
| $ZnCl_2$ | M/1000 | *T. gypseum* | —24 |
| $ZnCl_2$ | M/10000 | *T. gypseum* | —13 |
| $CdCl_2$ | M/100 | *T. rubrum* | —22 |
| $CdSO_4$ | M/100 | *T. rubrum* | +37 |
| $AgNO_3$ | M/100 | *T. rubrum* | —100 |
| $AgNO_3$ | M/10000 | *T. rubrum* | —43 |
| $HgCl_2$ | M/100 | *T. rubrum* | —74 |
| $HgCl_2$ | M/1000 | *T. rubrum* | —28 |

**The Effect of Certain Heavy Metal Ions on Oxygen Consumption:**— The effect of additions of dilute concentrations of certain inorganic water-soluble salts on the respiration of the dermatophytes has been examined by Nickerson and Chadwick. The following cations were among those included in the study: silver, mercury, zinc, and cadmium. As shown in Table 11, the first three of these proved to be inhibitory at concentrations as low as 1/10000 molar; cadmium had no pronounced effect on the respiratory rates of the organisms studied. A partial recovery in oxygen consumption followed the removal of M/100 $ZnCl_2$ from *E. floccosum;* an inhibition of 92-93% was changed to a fairly constant rate 59-65% lower than the control on washing out the zinc chloride and replacing the fungus in buffer solution. There was no recovery of *T. rubrum* from the 100% inhibition promoted by M/100 $AgNO_3$. Attempts to overcome the toxic effects of zinc salts by adding dilute concentrations of sodium thiosulfate, magnesium sulfate, or calcium chloride to zinc-poisoned preparations were unsuccessful. Reports of the success of such attempts are to be found in the literature and are frequently interpreted as indications of the mechanism of action of zinc salts. To date, however, there seems to be no convincing scheme of the mechanism of action of zinc (see the review by HEGSTED, MCKIBBIN and DRINKER, 1945). The same might be said of silver, for which no specific mode of action seems to have been uncovered. From the recent work of FILDES (1940) it seems clear that the action of mercury lies in its affinity for sulfhydryl (SH) groups; in view of the inhibition noted in Table 11, the dermatophytes would appear to employ active SH groups to a considerable extent in the mediation of their respiration. Heretofore, the activity of the mercuric ion against microorganisms has generally been credited to its power to precipitate proteins, but the demonstration that the mercuric ion also possesses marked affinity for sulfhydryl groups makes it probable that it is by

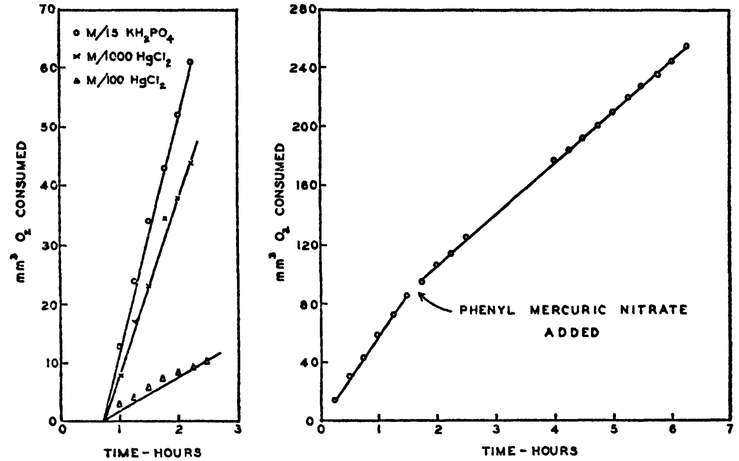


FIGURE 10. — *Effects of inorganic and organic mercury compounds on oxygen consumption by dermatophytes.*—Action of mercuric chloride (28 and 74% inhibitions) on *T. rubrum* (at left) and of M/10000 phenyl mercuric nitrate on *T. gypseum* (40% inhibition). Closely similar weights of fungus; both at 28° C. in an oxygen atmosphere; *p*H 4.5 in each case. (Previously unpublished figures.)

virtue of this property that the mercuric ion may interfere with metabolic processes in cells. This interpretation brings into fold the fact that the antibacterial effect of $Hg^{++}$ can be neutralized specifically by compounds containing a sulfhydryl group; this reversal can be effected even after long exposure of the microorganism to the mercuric ion, indicating the probable absence of a significant non-reversible, terminal process such as a denaturative precipitation. As DUBOS (1942) pointed out, the anti-sulfhydryl hypothesis accounts for some facts that were incompatible with the view that mercurial germicides act by virtue of their ability to precipitate proteins, an example being the case of some organic mercurials which, although less highly ionized than inorganic salts, are often more active against bacteria. It will be observed in Figure 10 that phenyl mercuric nitrate more effectively reduces respiration than an equivalent centration of mercuric chloride.

**Clinical Use of Inorganic Salts for Control of Fungus Infections:**— In view of the inhibitory effect of water soluble zinc salts on the respiration of the dermatophytes, DOLCE and NICKERSON (1947) undertook a clinical trial of zinc chloride in dilute solutions with superficial fungus infections. No effect was noticed on a few cases of infections of the scalp caused by *Microsporum Audouini* and the condition of high moisture generally associated with tinea pedis did not favor the use of zinc chloride solutions. In infections of the glabrous skin and in crural infections, there was some indication of usefulness for this salt. As the authors emphasized, the point of the work was not to add another compound to the long list of substances that have been employed in the treatment of fungus diseases but to put to trial compounds for which there was evidence on experimental grounds indicative of potential value.

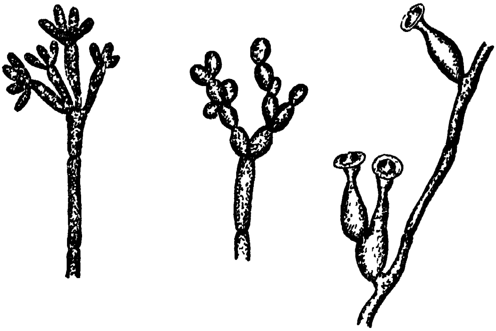
The use of respiratory studies as a metabolic bioassay method for securing information on the relative effectiveness of fungi-static chemical compounds under conditions approximating those prevailing *in situ* has been proposed by NICKERSON (1946). A more complete understanding of the physiology of the organisms causing superficial and systemic fungus diseases will be of material assistance in the control of such infections.

*Literature Cited:* —

1. BARKER, H. A. J. Cell. Comp. Physiol. *8:* 231, 1936.
2. BARRON, E. S. G. In Symposium on Respiratory Enzymes. Univ. Wisconsin Press, Madison, 1942, p. 262.
3. BERNHEIM, F. J. Bact. *44:* 533, 1942.
4. BERNSTEIN, D. E. Arch. Biochem. *3:* 445, 1944.
5. BOER, S. R. DE. Rec. trav. botan. néerland. *25:* 117, 1928.
6. BOREI, H. and S. LINDVALL. Biochem. Zeit. *316:* 160, 1943.
7. BURRIS, R. H. and P. W. WILSON. J. Cell. Comp. Physiol. *19:* 361, 1942.
8. BURRIS, R. H. and P. W. WILSON. Ann. Rev. Biochem. *14:* 685, 1945.
9. CHUDIAKOW, N. v. Landw. Jahrb. *23:* 391, 1894.
10. CLIFTON, C. E. Enzymologia *4:* 246, 1937.

11. Conant, N. F., D. S. Martin, D. T. Smith, R. D. Baker and J. L. Callaway. Manual of Clinical Mycology. Saunders, Philadelphia, 1945.
12. Cori, C. F. Biological Symposia *5:* 131, 1941.
13. Custers, M. Th. J. Onderzoekingen over het gistgeslacht *Brettanomyces.* Diss., Delft, 1940.
14. Dixon, M. Manometric Methods, 2d edition. Univ. Press, Cambridge, 1943.
15. Dolce, F. and W. J. Nickerson. Arch. Dermatol. Syphilol. *55:* 379, 1947.
16. Doudoroff, M. Enzymologia *9:* 59, 1940.
17. Doudoroff, M., W. Z. Hassid and H. A. Barker. Science *100:* 315, 1944.
18. Dubos, R. J. Ann. Rev. Biochem. *11:* 659, 1942.
19. Fernandes, D. S. Proc. Kon. Akad. v. Wet. Amsterdam *26:* 408, 1923.
20. Fildes, P. British J. Exptl. Path. *21:* 67, 1940.
21. Giesberger, G. Beitrage zur Kenntnis der Gattung *Spirillum* Ehrbg. Diss., Utrecht, 1936
22. Giese, A. C. J. Cell. Comp. Physiol. *20:* 35, 1942.
23. Giese, A. C. and E. L. Tatum. Arch. Biochem. *9:* 1, 1946.
24. Goddard, D. R. J. Infect. Diseases *54:* 149, 1934.
25. Goddard, D. R. in Höber, Physical Chemistry of Cells and Tissues. Blakiston, Philadelphia, 1946.
26. Goddard, D. R. and P. E. Smith. Plant Physiol. *13:* 241, 1938.
27. Gortner, R. A. Outlines of Biochemistry, 2d ed. Wiley & Sons, New York, 1938.
28. Green, D. E. Mechanisms of Biological Oxidation. Macmillan, New York, 1940.
29. Green, D. E. and P. K. Stumpf. Ann. Rev. Biochem. *13:* 1, 1944.
30. Haagen-Smit, A. J. and K. V. Thimann. J. Cell. Comp. Physiol. *11:* 389, 1938.
31. Hegsted, D. M., J. M. McKibbin and C. K. Drinker. U. S. Pub. Health Service, Pub. Health Rept. Suppl. No. 179, 1945.
32. Hotchkiss, R. D. Advances in Enzymology *4:* 153, 1943.
33. Klein, G. Handbuch der Pflanzenanalyse, vol. 4, p. 1259, Wien, 1933.
34. Kluyver, A. J. and L. H. C. Perquin. Biochem. Zeit. *266:* 68, 1933.
35. Kluyver, A. J. and M. Th. J. Custers. Anton v. Leeuwenhoek *6:* 121, 1940.
36. Lardy, H. A. and C. A. Elvehjem. Ann. Rev. Biochem. *14:* 1, 1945.
37. Lipman, F. Ann. Rev. Biochem. *12:* 1, 1943.
38. Martin, D. S., C. P. Jones, K. F. Yao and L. E. Lee. J. Bact. *34:* 99, 1937.
39. Martin, D. S. and C. P. Jones. J. Bact. *39:* 609, 1940.
40. McElroy, W. D. J. Cell. Comp. Physiol. *23:* 171, 1944.
41. Myrbäck, K. and E. Vasseur. Zeit. physiol. Chem. *277:* 171, 1943.
42. Nickerson, W. J. Science *103:* 484, 1946.
43. Nickerson, W. J. and W. R. Carroll. J. Cell. Comp. Physiol. *22:* 21, 1943.
44. Nickerson, W. J. and W. R. Carroll. Arch. Biochem. *7:* 257, 1945.
45. Nickerson, W. J. and J. B. Chadwick. Arch. Biochem. *10:* 81, 1946.
46. Peck, R. L. and C. R. Hauser. J. Am. Chem. Soc. *60:* 2599, 1938.
47. Peck, R. L. and C. R. Hauser. J. Biol. Chem. *134:* 403, 1940.
48. Pfeiffer, H. Biol. Revs. *4:* 1, 1929.
49. Pickett, M. J. and C. E. Clifton. J. Cell. Comp. Physiol. *21:* 77, 1943.
50. Raistrick, H. and W. Young. Phil. Trans. Roy. Soc. London, *B220:* 173, 1931.
51. Runnström, J. and K. Brandt. Arkiv f. Kemi *15A:* 1, 1941.
52. Schade, A. L. and K. V. Thimann. Am. J. Botany *27:* 659, 1940.
53. Scholander, P. F. and G. A. Edwards. Rev. Sci. Instr. *13:* 292, 1942.
54. Selzer, L. and J. P. Baumberger. J. Cell. Comp. Physiol. *19:* 281, 1942.
55. Sevag, M. G., R. A. Richardson and J. Henry. J. Bact. *49:* 129, 1945.
56. Spiegelman, S. and M. Nozawa. Arch. Biochem. *6:* 303, 1945.
57. Stier, T. J. B. Cold Spring Harbor Symposium Quant. Biology *7:* 385, 1939.
58. Stier, T. J. B. and J. N. Stannard. J. Gen. Physiol. *19:* 479, 1936.
59. Stier, T. J. B., M. I. Newton and H. Sprince. Science *89:* 85, 1939.

60. Stier, T. J. B. and C. F. MacIntyre. J. Cell. Comp. Physiol. *20:* 385, 1942
61. Symposium on Respiratory Enzymes. Univ. Wisconsin Press, Madison, 1942.
62. Tang, P. S. J. Cell. Comp. Physiol. *7:* 475, 1936.
63. Tang, P. S. J. Cell. Comp. Physiol. *8:* 109, 1936a.
64. Tate, P. Biol. Revs. *4:* 41, 1929a.
65. Tate, P. Parasitology *21:* 31, 1929b.
66. Umbreit, W. W., R. H. Burris and J. F. Stauffer. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Burgess, Minneapolis, 1945.
67. van Niel, C. B. and E. H. Anderson. J. Cell. Comp. Physiol. *17:* 49, 1941.
68. van Niel, C. B. and A. L. Cohen. J. Cell. Comp. Physiol. *20:* 95, 1942.
69. Welch, A. D. Physiol. Revs. *25:* 687, 1945.
70. Winzler, R. J. J. Cell. Comp. Physiol. *15:* 343, 1940.
71. Winzler, R. J. Science *99:* 327, 1944.
72. Winzler, R. J. and J. P. Baumberger. J. Cell. Comp. Physiol. *12:* 183, 1938.
73. Winzler, R. J., D. Burk and V. Du Vigneaud. Arch. Biochem. *5:* 25, 1944.
74. Wolf, F. T. and C. S. Shoup. Mycologia *35:* 192, 1943.



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